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Prime #: Contract entity: GTRC

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Project director(s):
ROGERS P H MECH ENGR (404)894-3235

Sponsor/division names: NAVY / OFC OF NAVAL RESEARCH
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Title: MODELS FOR THE DIRECTIONAL ACOUSTIC STARTLE REFLEX IN FISH

PROJECT ADMINISTRATION DATA

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Project Director ROGERS, PETER

Project Unit MECH ENGR

Sponsor NAVY/OFC OF NAVAL RESEARCH

Division Id 3314

Contract Number N00014-94-1-0328 Contract Entity GTRC

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Project File	Y

NOTE: Final Patent Questionnaire sent to PDPI

Progress Report for

Models for the Directional Acoustic Startle Reflex in Fish

Peter H. Rogers
Thomas N. Lewis

Georgia Institute of Technology

September 20, 1994

1. Overview of Scientific Progress

The experimental investigation of the acoustic Mauthner reflex will involve observations of the fish's responses to high amplitude impulsive monopole and dipole sources with varying positions, orientations, waveforms, and polarity in a large tank. Recent efforts have been directed at characterizing the ambient noise levels in the tank, determining its modal structure, and developing our ability to control the sound field using multiple projectors. The components for the video monitoring system are also being assembled, integrated, and tested. Also our study on the detection of scattered ambient noise by the goldfish has been completed. The study demonstrated that the goldfish could detect scattered noise at a biologically relevant range.

2. Accomplishments

The principle direction of our research is to determine how the auditory systems of fish function and how they are used to help the fish survive in the underwater environment. We have hypothesized that one fish could perceive nearby fish by recognizing the scattering of ambient noise by the other's swim bladders. Recently completed studies (Lewis, 1994) tested the basic premises of this hypothesis by (1) determining the characteristics of noise scattered by the swim bladder of two species of fish and (2) measuring the ability of the goldfish to discriminate this scattered ambient noise signal from the background ambient noise. The results indicated that the detection range was on the order of the length of the scattering fish. Therefore, this type of perception could be biologically relevant to a prey fish needing to detect a much larger preditor fish. This type of perception could also be useful for the Navy, as they work in the same noisy underwater environment and have the need to locate and identify resonant structures, such as mines, in that environment.

The results from one detection threshold study are shown in Fig. 1. The pressure signal-to-noise was independent of range, implying that this otophysan fish (goldfish) senses acoustic pressure. If the fish's auditory system detects pressure, then the sense is non directional. A attempt to demonstrate directional hearing using identical sources differing only in bearing failed. This result is in potential conflict with previous studies on the directionality of the acoustically mediated Mauthner reflex and on the demonstrated directionality of hearing in the nonotophysan cod. This is a continuation of work sponsored by the ONR Marine Biology program.

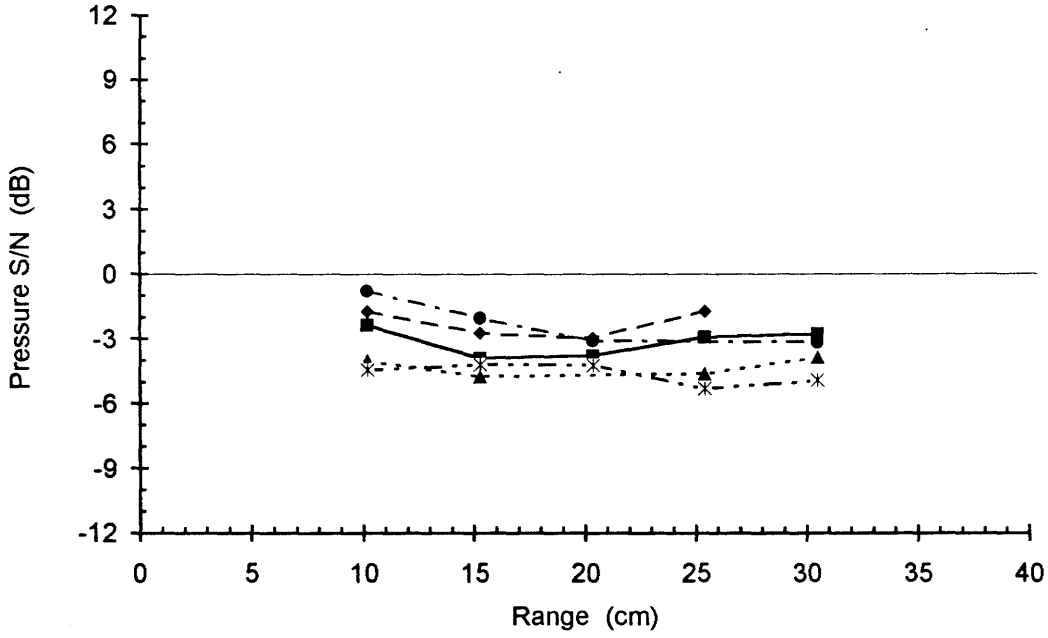

Fig. 1. Data for the detection threshold versus range (distance from the scatterer to the subject) study. The task was to detect scattered ambient noise in the ambient noise field. The noise was broadband white noise (200 to 1200 Hz); the signal the same noise bandpass filtered (Q = 5.6) around 750 Hz. The S/N is the ratio of the peak of the scattered noise signal to the average broadband noise level. Different symbols represent different subjects.

3. Productivity Report

a) 0, b) 0, c) 0,

d) Lewis, T.N. (1994). "Detection of scattered ambient noise by fish," Ph.D. Thesis, George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA.

e) 0, f) 0,

g) Rogers, P.H., G.W. Caille, and T.N. Lewis (1994). "Response of the lungs to low frequency underwater sound," Summary Meeting on the Effects of Low-Frequency Water-Borne Sound on Divers, Naval Submarine Base New London, Groton, CT, June 29, 1994.

h) C - Lewis, T.N., J.S. Martin, and P.H. Rogers (1994). "Measurement of the vibrational response of porcine lungs to low-frequency underwater sound," J. Acoust. Soc. Am. 95, 2aBV4.

C - Lewis, T.N. and P.H. Rogers (1994). "Directional hearing in the goldfish (Carassius auratus)," J. Acoust. Soc. Am. 95, 3aAB6.

i) The Non-Invasive Vibration Amplitude Measurement System (NIVAMS), initially developed to measure the resonance characteristics of fish swim bladders, was adapted to determine the effect of low frequency underwater sound on the lungs of swimmers and divers. This project, initiated by the Space and Naval Warfare Systems Command (Codes PMW 182, PMW 182PA, and PD80P2), was directed by the Naval Submarine Medical Research Laboratory (CAPT P.K. Weathersby, CO) in response to immediate operational concerns for the safe use of the LFA system. During this yearlong investigation, we used the NIVAMS to determine the frequency response of the lungs of divers at the water surface and at depth. In vivo measurements on human subjects were carried out at the Navy's Ocean Simulation Facility in conjunction with the Navy Experimental Diving Unit (CDN Bert Marsh, CO) in Panama City, FL. The results from this study were used to help set guidelines for the Navy's use of high intensity, low frequency underwater sound.

j) 2 graduate students and 2 undergraduates

k) Peter H. Rogers - Appointed to the Rae and Frank H. Neely Chair in Mechanical Engineering in The George W. Woodruff School of Mechanical Engineering at the Georgia Institute of Technology

Thomas N. Lewis - Graduated with a Ph.D. in Mechanical Engineering from the Georgia Institute of Technology

l) Macintosh 660AV computer system

Progress Report for

Models for the Directional Acoustic Startle Reflex in Fish

Peter H. Rogers
Thomas N. Lewis

Georgia Institute of Technology

August 15, 1995

1. Overview of Scientific Progress

The experimental investigation of the acoustic Mauthner reflex will involve observations of the fish's responses to high amplitude impulsive monopole and dipole sources with varying positions, orientations, waveforms, and polarity in a large tank. A baseline study of normal fish is in progress. Next, animal preparations with modified sensory pathways will be studied. Another effort has been directed toward identifying a biologically relevant acoustic source which elicits the startle response. Also preliminary work on the detection of scattered ambient noise by a non-ostariophysan has been initiated.

2. Accomplishments

The most interesting accomplishment over the past year has involved the identification of a potentially relevant acoustic stimulus to elicit the startle reflex in fish. We have been studying the startle response without having previously determined a source of the acoustic stimulus. Understanding the physics of sound generation by a relevant source may help us understand the mechanisms that a fish uses to detect and respond to that source.

The strike of an attacking predator involves a sudden forward jerk and a sucking motion from the mouth of the fish. These motions radiate sound. If the prey fish can detect this radiated acoustic field and respond to it using the startle reflex to escape, then the acoustically mediated startle reflex is biologically relevant to fish.

The strike motion was modeled by a suddenly accelerating rigid body. Acoustic theory was used to estimate the acoustic field radiated by this model. A literature search revealed that an attacking fish can accelerate at 4 g, even up to 10 g for one species. Accelerations at this rate radiate sound fields in the frequency and amplitude ranges that may elicit the startle response.

A preliminary attempt was made to measure the sound generated by an attacking fish. A predator fish was housed in an aquarium in which a hydrophone was mounted. A video camera was used to monitor the movements of the predator as well as the acoustic pressure detected by the hydrophone during feeding of live fish. Preliminary analysis of the hydrophone recordings indicated that sound generated by the striking predator was within the hearing range of many fish. Whether this sound is capable of eliciting the startle response remains to be determined.

3. Productivity Report

a) 0

b) Lewis, T.N. and P.H. Rogers (1996). "The vibrational response of a single-chambered fish swim bladder to low frequency sound," ICES J. Mar. Sci., in press.

c) 0, d) 0, e) 0, f) 0, g) 0

h) C - Lewis, T.N, and P.H. Rogers (1995). "The vibrational response of single-chambered and two-chambered fish swim bladders to low frequency sound," ICES International Symposium on Fisheries and Plankton Acoustics, Aberdeen, Scotland.

i) The Non-Invasive Vibration Amplitude Measurement System (NIVAMS), initially developed to measure the resonance characteristics of fish swim bladders, was adapted to determine the effect of low frequency underwater sound on the lungs of swimmers and divers. This project, initiated by the Space and Naval Warfare Systems Command (Codes PMW 182, PMW 182PA, and PD80P2), was directed by the Naval Submarine Medical Research Laboratory (Lt C. Steevens, POC) in response to immediate operational concerns for the safe use of the present LFA and future LLFA systems. Our previous work on high intensity sound exposure of fish was applicable to the study on sound exposure of divers by identifying the vestibular system as a potential source of concern. The results from this study will be used to help set guidelines for the Navy's use of high intensity, low frequency underwater sound.

j) 1 graduate student

k)

l) 1 (WIN 486 PCI computer system
National Instruments AT-MIO-16E-10 Data Acquisition board

No. of Undergraduates: 0

ONR ANNUAL PRODUCTIVITY REPORT, 15 SEP 1994 TO 14 SEPT 1995

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Project Title: Models for the Directional Acoustic Startle Reflex in Fish

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** Attach list of papers and other publications with full citation.**

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** Attach title and brief description of patents/inventions, if any.**

Number of Presentations:

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Contributed: 1

Trainee Data:

	Total	Female	Male	Minority	Non-US Citizens
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No. of Postdoctorals:	0	0	0	0	0
No. of Undergraduates:	0	0	0	0	0

Publications

Lewis, T.N. and P.H. Rogers (1996). "The vibrational response of a single-chambered fish swim bladder to low frequency sound," ICES J. Mar. Sci., in press.

Final Report for

Navy Contract N00014-94-1-0328

Models for the Directional Acoustic Startle Reflex in Fish

Principal Investigator

Peter H. Rogers

George W. Woodruff School of Mechanical Engineering

Co-Principal Investigator

Thomas N. Lewis

George W. Woodruff School of Mechanical Engineering

Georgia Institute of Technology

September 26, 1997

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13. ABSTRACT (Maximum 200 words)

Fish exhibit a characteristic Mauthner-initiated startle response to sudden acoustic stimuli. This escape response is thought to be triggered by predatory attacks since an acoustic wave is launched ahead of a striking predator. The nature of acoustic stimuli which elicit the startle response was examined in free swimming goldfish. The results showed that a threshold level of acoustic acceleration (-30 dB re: 1 m/s2) was required to elicit a startle and that the threshold was invariant of bearing to the source. Near threshold, the fish turned in a random direction, but as the stimulus level increased, the fish was more likely to turn away from the source. The data indicated that the lateral component of the acoustic acceleration was the relevant parameter determining directionality. A proposed model uses the utricle to detect acoustic acceleration directly, the saccule to detect acoustic pressure indirectly, and logical wiring to perform the neural computation which explains the fish's behavioral performance.

14. SUBJECT TERMS
startle reflex, underwater hearing, fish, auditory computation, predator-prey interaction

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Abstract

Fish exhibit a characteristic Mauthner-initiated startle response to sudden acoustic stimuli. This escape response is thought to be triggered by predatory attacks since an acoustic wave is launched ahead of a striking predator. The nature of acoustic stimuli which elicit the startle response was examined in free swimming goldfish. The results showed that a threshold level of acoustic acceleration (-30 dB re: 1 m/s^2), not pressure, was required to elicit a startle and that the threshold was invariant of bearing to the source. Near threshold, the fish turned in a random direction, but as the stimulus level increased, the fish was more likely to turn away from the source. The data indicated that the lateral component of the acoustic acceleration was the relevant parameter determining directionality. A proposed model uses the utricle to detect acoustic acceleration directly, the saccule to detect acoustic pressure indirectly, and logical wiring to perform the neural computation which explains the fish's behavioral performance.

Introduction

Fish exhibit a characteristic Mauthner-initiated startle response to sudden acoustic stimuli [Eaton et al. 1977, Zottoli, 1977]. This type of escape response can be triggered by predatory attacks [Webb, 1986]. In one mode of attack, the stationary predator lunges forward toward the center of mass of the solitary target while opening its mouth to suck in the prey [Webb and Skadsen, 1986]. Launched ahead of the rapidly accelerating predator is an acoustic wave which propagates at the sound speed of the media (1500 m/s). The prey is sometimes able to detect the striking predator, determine its direction, and respond by rapidly turning and swimming away. Whether the prey is responding to the acoustic signal or whether the response is multimodal (vision and/or the lateral line may play a role) is not understood.

The escape response consists of a sudden turn and a rapid acceleration [Eaton and Emberley, 1991]. The sudden turn involves a major contraction of the musculature on one side of the fish, bending both the head and tail to one side to a C-like shape. This coordinated motion is triggered by one of the Mauthner cells, a bilateral pair of neurons whose dendrites and soma receive various sensory inputs and whose axons descend in the spinal cord and synapse with the primary motorneurons along the side of the trunk opposite the soma. The initial turn is followed by acceleration toward the final escape trajectory.

The acoustic startle reflex is thought to be initiated by the fish's inner ear [Moulton and Dixon, 1967]. These otolithic organs act as inertial accelerometers, responding to the motion of the fish caused by the motion of the surrounding water [Fay, 1984]. Acoustic particle motion can be detected directly by the inner ear, giving information about the source direction. Acoustic pressure is detected indirectly, either through reradiation by the swimbladder or through a more direct coupling such as the Weberian ossicles.

In the goldfish, the Mauthner cell fires within about 4 msec after the onset of a low frequency acoustic stimulus [Eaton et al., 1995]. Since the startle response happens quickly after the initiation of the sound, the concept of acoustic frequency for the stimulus loses meaning. One cycle of a 100 Hz wave takes 10 msec to complete. If the startle response is initiated in less than the period, then the response is not dependent upon any periodicity of the signal. Therefore, tonic responses of the nervous system are more influential than phasic responses.

The purpose of this research was to examine the nature of acoustic stimuli which elicit the startle response. A set of experiments were designed and carried out to measure thresholds

of startle response to several acoustic signals. Individual fish were placed in the center of a large water tank. A large tank was necessary so that the response of the fish would be only to the direct signal from the transducer, not any wall reflections. The free swimming fish was exposed to the acoustic stimulus, and its responses were recorded and graded for existence and direction. From this data, thresholds and correctness percentages (correct responses were directed away from the acoustic source) were calculated relating to the parameters of the incident acoustic wave on the fish.

The information on quantifying threshold levels which elicit response and the directionality of those responses are useful in two ways. First, the original hypothesis that prey fish startle in response to the acoustic wave launched by an attacking predator can be tested by comparing startle thresholds with strike signatures. Future research is needed to characterize predatory strike acoustic signatures. Second, understanding the threshold parameters and directionality have yielded clues to the functionality of the auditory system and the connection between it and the Mauthner cell. A proposed model for the directional startle response has been tested and refined which describes how the ear is able to detect and interpret acoustic waves in order to directionalize and respond to sources.

Methods

The test subjects were 10 goldfish (*Carassius auratus*) ranging in weight from 0.91 gr. to 27.0 gr. mass, obtained locally, and kept in community tanks for the duration of the studies. These experiments were performed under the auspices of the IACUC of the Georgia Institute of Technology.

Startle thresholds to acoustic stimulation were obtained by monitoring the response of individual subjects to a series of acoustic stimuli varying only in amplitude. The individual trials were graded for a binary response (yes-no) and direction of the head and tail motion of the initial C-shaped bend (C-start). Occasionally, a fish would appear to respond to an acoustic stimulus by a non-characteristic motion for which the latency was much longer (>100 msec) than the characteristic C-start. This response was graded as a weak response, but was interpreted as not a C-start. The data from each series of trials was fit to a standard psychometric function. Threshold was defined as the sound pressure level which yielded a 50% probability of response.

During the experimental trials, the fish was kept in a 2 gallon size Ziplock® brand freezer bag filled with water from the fish's home aquarium. The plastic bag was virtually transparent underwater, both optically and acoustically, except the visible midline seam and zipper. When filled, the bag contained a volume of water nominally 33 x 28 x 7 cm deep. The subject swam freely within this volume. All air bubbles were removed from the bag. The filled bag was sandwiched horizontally in an adjustable frame between two parallel Lexan® plates. A thin white rubber sheet was placed between the bag and the lower plate to serve as a contrasting background for the video. A rectangular grid was marked on this rubber sheet to aid in grading the responses. The frame was then lowered to the center of a 32,000 gallon cylindrical (5.8 m dia x 4.1 m deep) acoustic tank and hung by ropes [Fig. 1]. Both the acoustic tank and all home aquarium were maintained at 25°C. Suspended next to the frame in the tank was the acoustic source. No attempt was made to visually obscure the source location from the subject. Only one source was used.

The ideal stimulus would have been one that mimicked the acoustic signal generated by an attacking predator. For a linearly accelerating object toward the prey, this would consist of an initial positive pressure pulse. Stimulus signals to mimic this were created by a data acquisition board in a personal computer, amplified, and projected by a USRD J-9 underwater sound transducer. The transducer was oriented pointed toward the frame holding the fish and

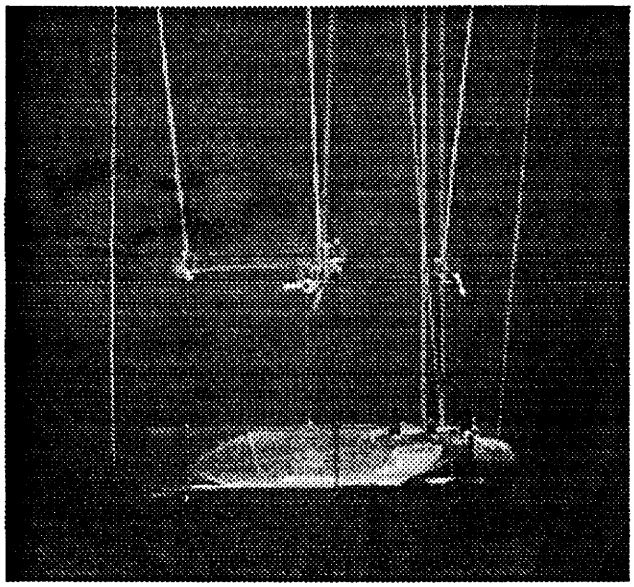

Figure 1. Side view of the goldfish in the bag within the frame suspended in the acoustic tank. The J-9 acoustic source is located behind and to the right of the frame.

at the same depth, with the piston face located 0.43 m from the center of the bag. The numerically generated signal was such that the initial positive pressure pulse was larger than the following negative portion of the acoustic wave.

Because of the response dynamics of the J-9, only a limited range of signals could be generated. Figure 2 shows the stimulus signals that were used. The parameter varied between the different pressure pulses is rise time, as measured as the time interval from 10% to 90% of the maximum signal level of the initial rise of the positive pressure pulse (Fig. 3). This stimulus system's performance was limited at long rise times as the relative amplitude of the peak positive pulse to the subsequent peak negative pulse decreased with increasing rise time. The performance was limited at shorter rise times by its inability to reproduce the higher frequency components. Although shorter rise time electrical signals could be generated, the resultant acoustic signals did not change.

The ringing after the initial pressure pulse is due to multiple reflections from the surface, bottom, and walls of the tank. Since the source and receiver were at mid depth, the air surface and cork bottom were both 2.03 meters away, so the inverted reflections arrived 2.7 msec after the direct signal from the source. The circular tank is free standing (air-backed), so the inverted reflection from the walls 2.90 meters away arrives 3.9 msec after the direct signal.

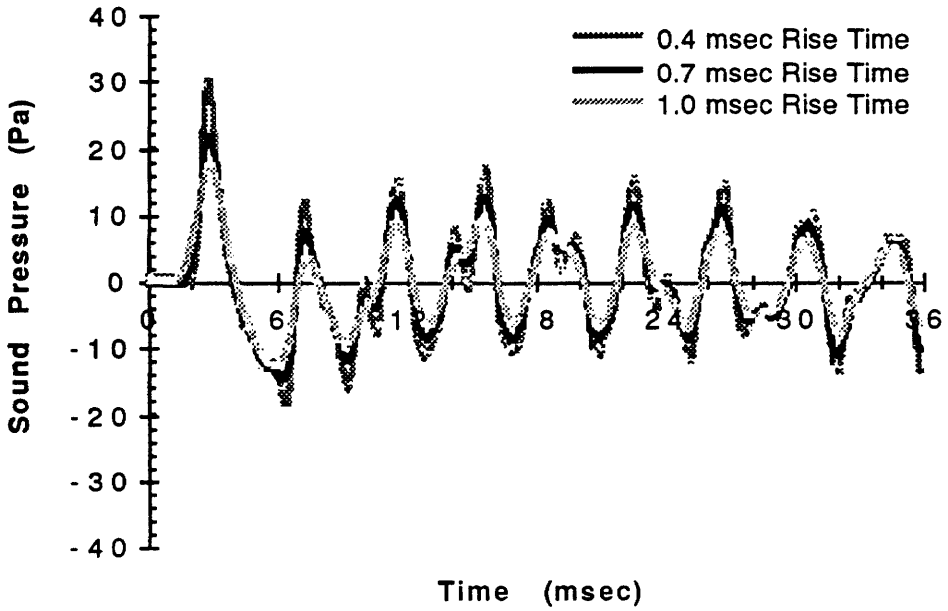

Figure 2. Acoustic pressure stimuli used in the startle experiments. Only the initial pulse comes directly from the source; the rest is due to reverberation within the tank.

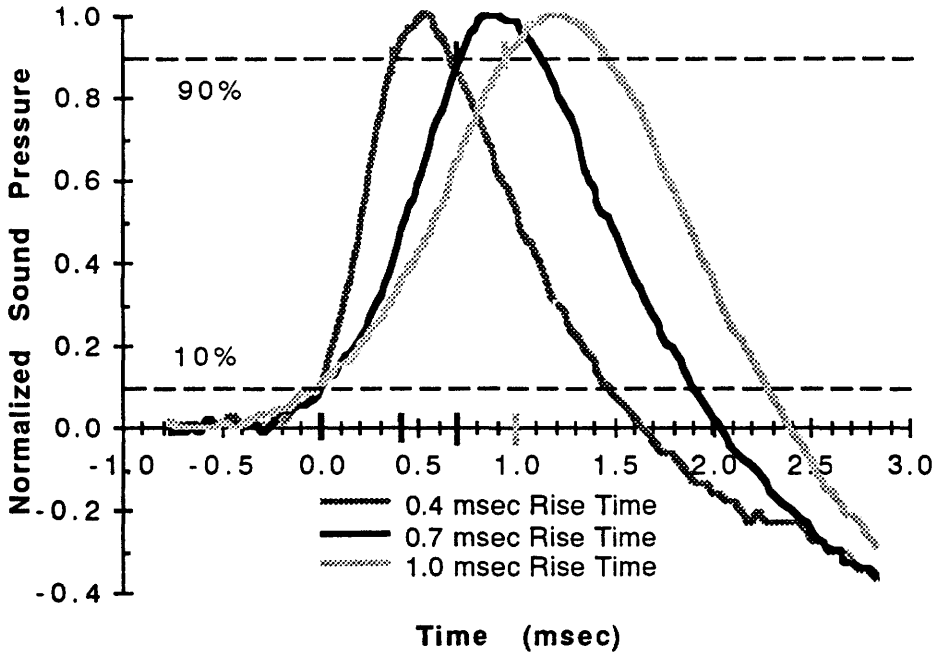

Figure 3. Measurement of rise time for the pressure pulse signals.

The assumption was made that the subject responded (or didn't) to the direct signal before the arrival of any reflected signal.

The acoustic system was calibrated by placing a B&K 8103 hydrophone in the tank in a location representing the center of the frame holding the fish. The output of the hydrophone was monitored and recorded on a Tektronics 2430A oscilloscope. One check was performed comparing the acoustic signals with and without the fish frame and freezer bag. Since the differences between the two signals were negligible, all further calibrations were performed without the frame.

The response of the fish was monitored by a video camera positioned to view across the top of the acoustic tank [Fig. 4]. A mirror was placed over the frame at a 45° angle so that the fish could be seen from the top. An oscilloscope was placed on the other end of the tank, such that the viewfinder of the camera contained the mirror with the top view of the subject and the display of the oscilloscope. The oscilloscope was triggered by the acoustic signal, so a trace would appear upon presentation of the stimulus. The oscilloscope was shielded so that there was no direct visual path between the display and the subject. The video signal from the

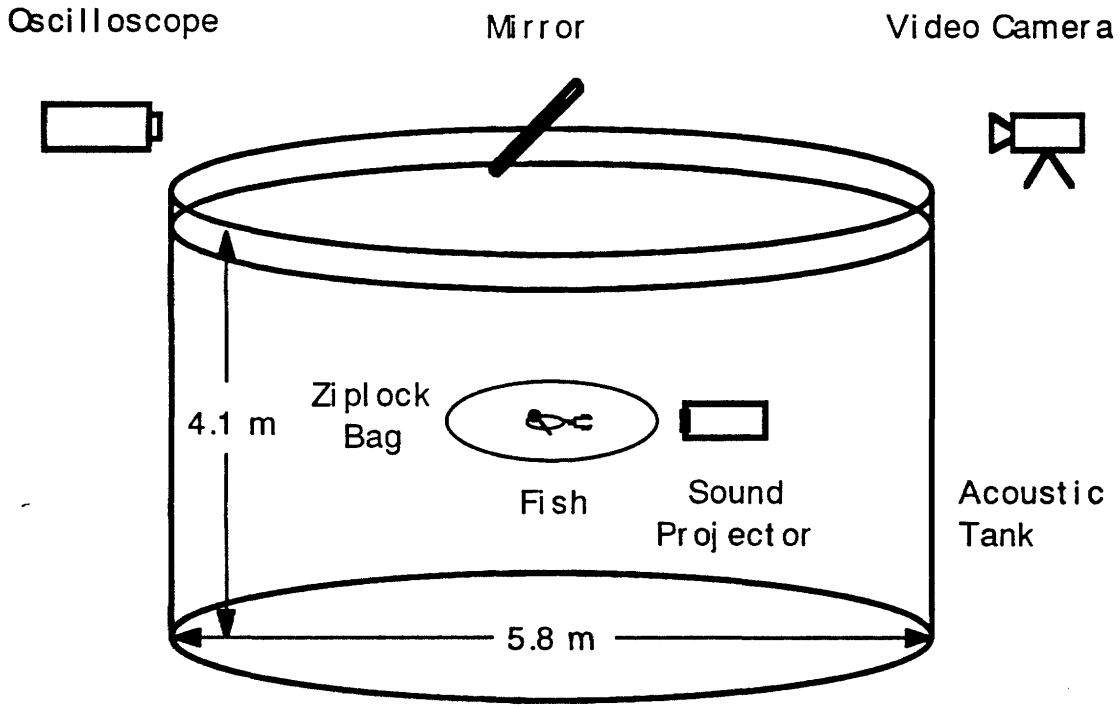

Figure 4. Schematic of key components for startle experiments.

camera was input into a VCR and a monitor on the control deck. The controls for the VCR were wired into the data acquisition board of the control computer. Because this auxiliary VCR was used to record the video signal, the camera, which was visible to the subject above through the mirror, was always in the same state (i.e. no flashing lights when recording).

The experiment was controlled by a microcomputer running a custom LabView (National Instruments) program. Individual trials were sequenced as follows:

1. The computer would select the next stimulus level and digitally create the signal. The time and the stimulus level were recorded in a computer datafile.
2. The recording VCR was enabled. Then there was a wait of 10 seconds plus a random wait of between 0 and 5 seconds.
3. The acoustic stimulus was presented. The oscilloscope on top of the acoustic tank was triggered simultaneously.
4. There was another wait of 5 seconds and the recording VCR was disabled.

The computer would randomly select an intertrial interval of between 9 min. 30 sec. and 10 min. 30 sec.

Since the trials were not graded in real-time, the method of constant stimuli was used to determine thresholds. Most experiments consisted of six stimulus levels plus catch trials (trials where the stimulus level was zero). Each stimulus level was presented eight times, for a total of 56 trials [Fig. 5]. Through experience, the stimulus level increment was chosen to be five dB. Since the threshold wasn't known a priori for a novel stimulus to a subject, threshold level was estimated by trial-and-error. These trials also served as system checks. The average intertrial interval was chosen to be ten minutes.

Although this was the final paradigm, not all trial series were run with these parameters. The intertrial interval was varied between series ranging from 8 to 15 minutes. This interval was necessary to prevent habituation to the signal, as the subject would discontinue responding to stimuli when the interval was on the order of one minute. Habituation would have been apparent as a decrease in response threshold for a given stimulus amplitude with trial number. Also the number of trials per stimulus was varied from 6 to 10, the higher number to test for habituation, and the lower as a compromise due to total time duration constraints. With the parameters as stated in the previous paragraph, a trial series would last over ten hours, including a 30 minutes wait after the subject was lowered to depth and couple of trials to estimate threshold and set the experimental stimulus levels.

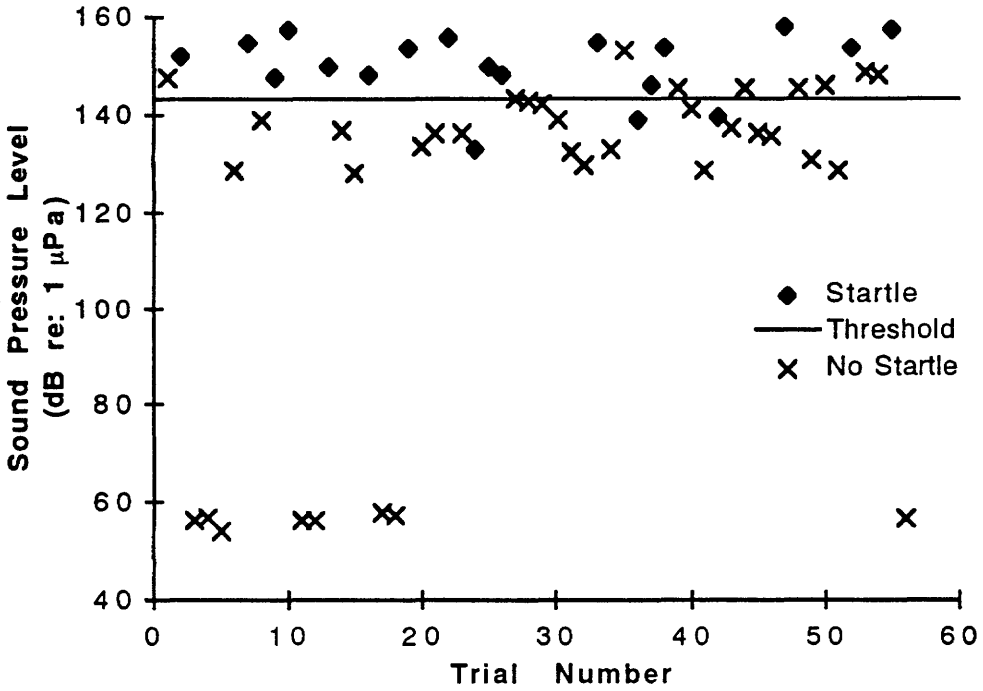

Figure 5. Example of a threshold measurement series. The ordinate is the peak sound pressure level at the fish. The trials with levels around 60 are catch trials (no stimulus).

Because sound pressure level is inversely proportional to the range from the source, the pressure field was not uniform in the horizontal plane. Therefore, it was necessary to know the location of the fish relative to the transducer to accurately determine the pressure to which the fish responded. The x-axis was placed along the midline of the acoustic source [Fig. 6].

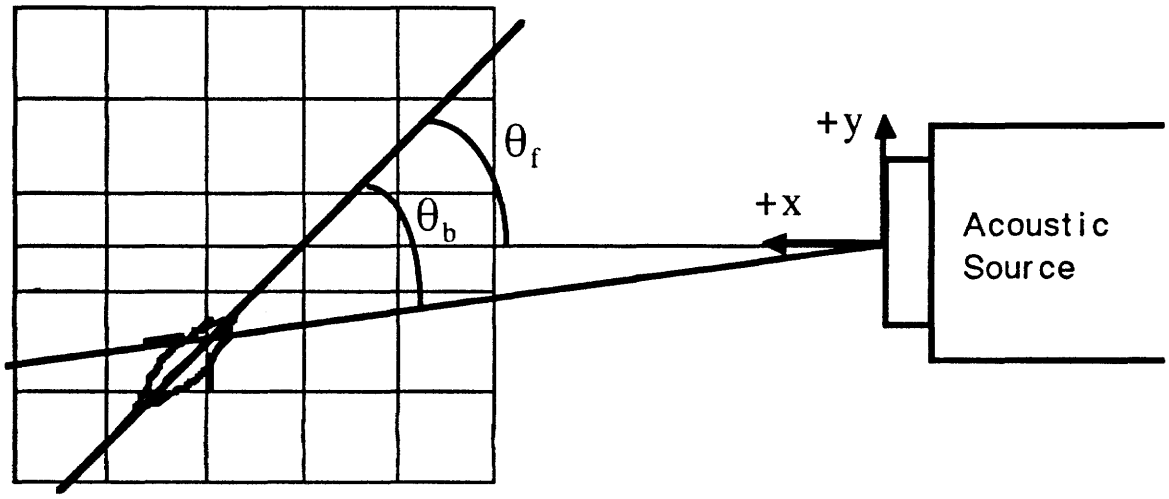

Figure 6. Location and orientation of fish relative to acoustic source.

The location of the fish was determined by measuring the position of the midline of the widest portion of the subject's body (at the insertion of the pectoral fins) in this coordinate system. The angular orientation of the subject, θ_f was measured between a line connecting this point and the rostral tip of the subject's mouth and the x-axis. The bearing of the transducer, θ_b was the angle between this vector and the vector from the fish to the center of the face of the transducer.

The tapes contained series of 15 sec. video clips containing individual trials. The image screen contained, on one side, the top view of the subject, and on the other side, the display of the oscilloscope [Fig. 7]. The internal clock of the video camera was visible at the bottom. The videos were graded without knowledge of the stimulus level.

The grader would run the tape of each trial and note the subjects response to the stimulus. The onset of the stimulus was coincident with the trigger of the trace on the oscilloscope. The trace on the oscilloscope was a flat line, not the stimulus signal, so it did not convey any amplitude information. Since the timebase of the oscilloscope was set to 10 msec/div, the trace would be visible in 4 consecutive frames (standard video is 30 frames/sec, so the interframe interval is 33.3 msec). As the stimulus was not synchronized with the video, the first post stimulus frame would occur randomly between 0 and 33.3 msec after the stimulus. The C-start occurs with an onset latency of about 20 msec, so it appeared in the first or second post-stimulus frame.

The grader would view each trial for the following information:

1. Time recorded on video tape.
2. Activity before stimulus: still (not moving), moving (gliding), or active (swimming).
3. Location and angle of position before stimulus: x-position, y-position, and Cartesian angle, θ_f.
4. Response: no, weak (there appeared to be a behavioral response but it occurred over 100 msec after the stimulus), or strong (characteristic C-start within 60 msec of stimulus).
5. Direction of response: to the subject's left side (counter clockwise) or right side (clockwise) of its initial position.
6. Position 7 frames (201-234 msec) after stimulus.

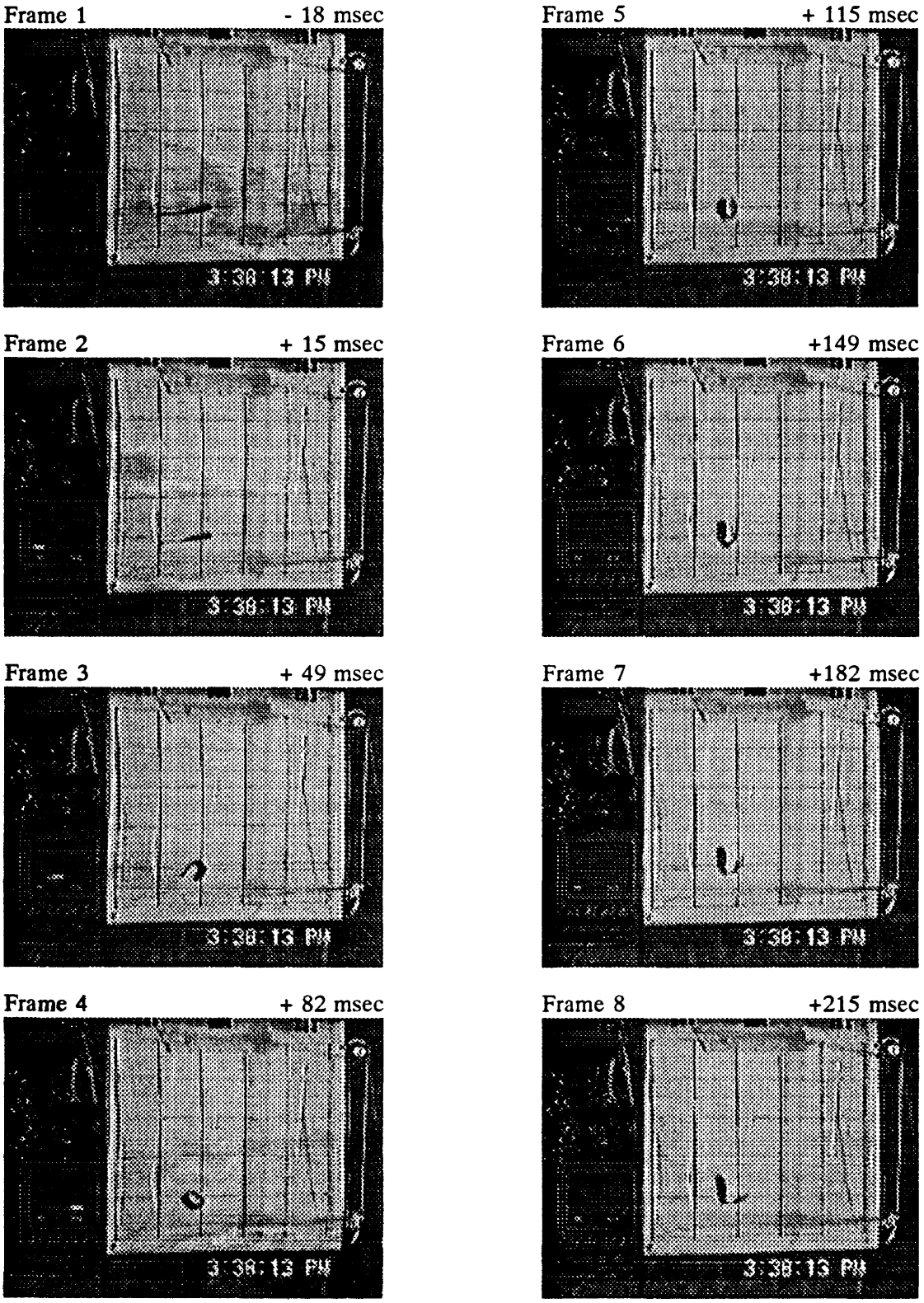

Figure 7. The video frame sequence from a startle trial. The subject (lower center) is oriented toward the source (offpage, center right). The trace on the oscilloscope (lower left) is triggered on the acoustic stimulus. The time is post-stimulus.

The reviewing VCR had freeze frame and single frame stepping capabilities to allow the grader to identify the last pre-stimulus frame for collecting the information for item 3. The characteristics for the C-start were defined as illustrated in Eaton and Emberley (1991) as the stage 1 component of response. This involves a major contraction of the musculature of only one side of the body, forming a C-like shape with the head and tail bent to one side. This response could be delineated even while the subject was swimming.

Figure 7 shows a sequence of frames from one trial. The oscilloscope is in the lower left corner of the frames. The right three-quarters of the frame contain the mirror viewing the fish from the top. The fish is located in the lower left corner of the grid, with its head facing to the right. The sound projector is not shown, but is along the midline of the grid to the right. The fish is probably facing it directly.

Frame 1 of Figure 7 is the final pre-stimulus frame, showing the subject's initial position. The fish had been still. The trace in frame 2 indicates the stimulus had been presented a little less than 20 msec before. The fish has performed a C-start by frame 3, 33.3 msec after frame 2, turning to its right. In this atypical case, the subject continued turning through 270 degrees, facing to the left of its initial position. Notice that further movements were much less dramatic than the C-start. Figure 8 shows the location of the fish for the individual trials of the series for the data in Figure 5.

After grading, the data was input to a spreadsheet and collated with the data file containing the signal level information. The sound pressure level at the subject and the bearing to the source were calculated. Then the directional response was compared to the 'correct' response, which was defined as a turn away from the acoustic source. Any trial where the subject was pointed toward the transducer ($-10° < \theta_b < 10°$) or away from the transducer ($\theta_b < -170°$; $\theta_b > 170°$) was not judged on directional correctness, as this range was within measurement error. Figure 9 shows the bearing of the fish to the source for the data from Figure 5.

Psychometric functions typically define a relationship between the probability of a response and the stimulus level. Experiments are performed with a number of trials at the same stimulus level to obtain a percent response at that level. The combined results from several stimulus levels are fit to the psychometric function. In this study, however, the subjects were free swimming in a nonuniform sound field, so that each trial could easily result in differing

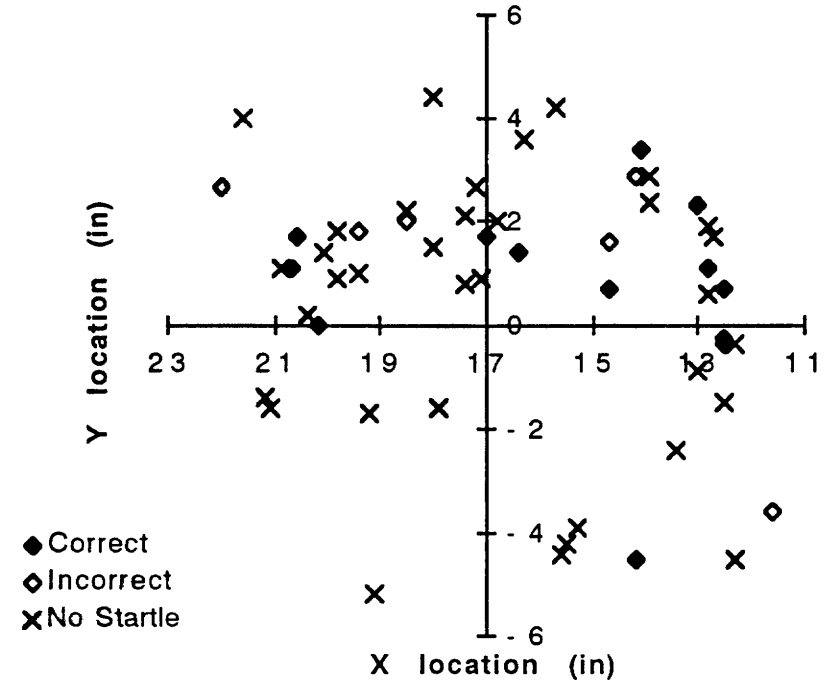

Figure 8. Response for each trial as a function of pre-stimulus location. This is the same trial series data from Figure 5.

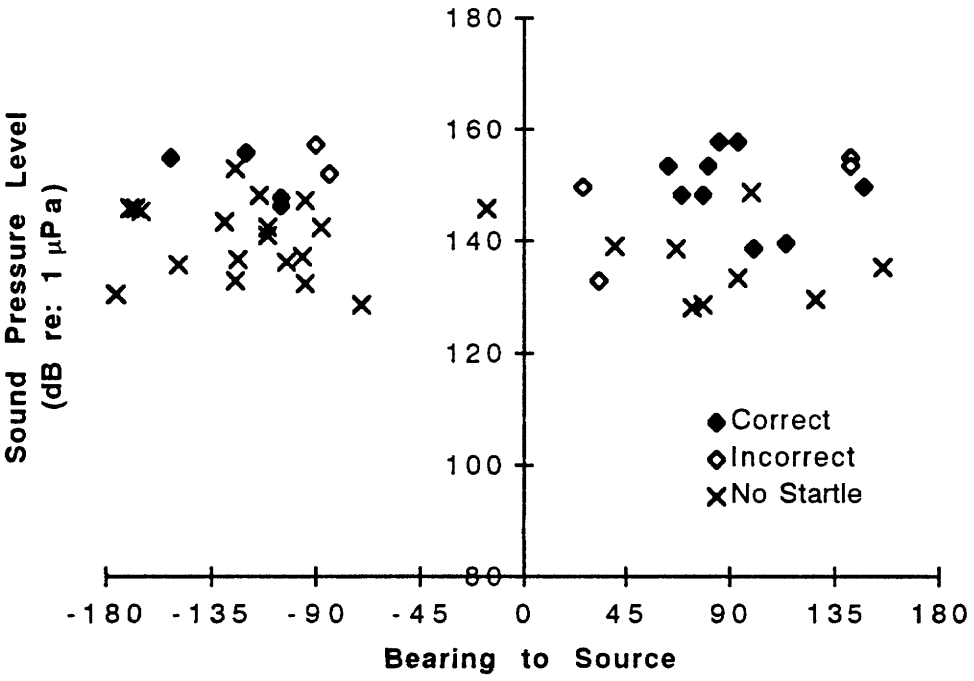

Figure 9. Response for each trial as a function of bearing to source. This is the same trial series data from Figure 5.

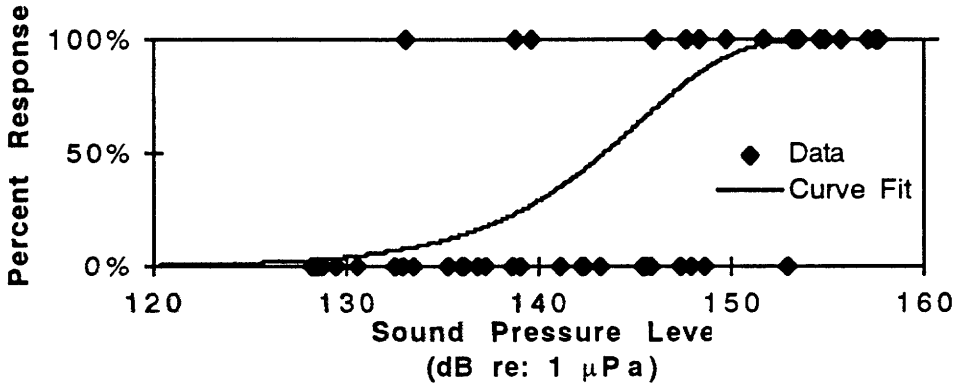

Figure 10. The curve fit of the Weibull function to the data from Figure 5. The peak sound pressure level at 50% response on the Weibull function was 143.4 dB.

stimulus levels at the subject. Therefore, the percent response at each stimulus level was 100 or 0 percent.

An example of a threshold series is shown in Figure 10. The data, not including catch trials, were then fit to the Weibull function

$$P(c) = (1 - e^{-(p_s/p_t)^{2\beta}}) * 100 \qquad (1)$$

where P(c) is percent response, p_s is the stimulus pressure, p_t is the pressure level at 63.2 percent response, and β is a parameter proportional to the slope at the mid range of the function (Fay and Coombs, 1992). The Weibull function has been shown to well summarize psychometric functions in human hearing. The curve fits were performed using the fmin function in MATLAB with the free parameters p_t and β. A normalized residual was calculated as the sum of the squares of the difference between the predicted percent response and the data for each data point, normalized by the number of data points. The best fit curve has the lowest residual. From the two free parameters, the sound pressure level for 50 percent response was then calculated.

An attempt was made to startle two oscars (*Astronotus oscellatus*). For the acoustic signals generated (up to 162 dB), the oscars did not startle.

Results

79 trial series were obtained on the 10 goldfish used as subjects. Of these 79 series, 34 were considered invalid for various reasons, including technical problems with the apparatus, incorrect stimulus range settings resulting in too many or too few responses, or data sets where the trials were ungradable. The 45 remaining trial series on five of the subjects contained 2,466 gradable trials. There were no C-starts to the sham stimulus during any of the 338 catch trials.

The results of the curve fits to the Weibull function are given in Table 1 and Figure 11. The first column gives the date the trial series was started. The data was collected over three periods: Nov. '95, Dec. '95, and Apr. '96, but results were only used from the second two periods. The digit past the decimal point discriminates multiple series run on the same day. The weights for the subjects were collected after each set of series were run. Curve fits were calculated in linear variables and converted to dB. Slopes of 10.0 indicate that the best fit function was a step function, where the percent response was 100% above threshold and 0% below.

One feature of this experimental apparatus was that the subject had a tendency to be positioned near boundaries. The bag constraining the fish was about 11 inches wide (x-direction) and about 13 inches long. For many of the trials, the subject was located near the wall and may have influenced the direction of the response. Eaton and Emberley (1991) found in their ball drop studies that when starting near a wall, the fish used an escape route that was not predictable from the stimulus angle. Another potential bias was that the acoustic source was always visible to the subject. The results on the directionality of the response are given with these caveats.

"Correct" responses were C-starts away from the source and "Incorrect" toward the source. Figure 12 is the data from the last column from Table 1 plotted as a function of fish mass. There is no apparent pattern to the response, either as a function of mass or rise time. Considering all of the 874 trials where the subject startled independently, the responses were 59.0% correct.

Table 1. Curve fit for the acoustic pressure threshold series to the Weibull function.

Date (yymmdd.n)	Fish Name	Weight (g)	Rise Time (msec)	Pressure Threshold (dB)	Slope (β)	% Correct
951219.3	Bigger Guy	2.5	0.4	147.6	0.54	60.0
960414.2	Bigger Guy	3.2	0.4	145.7	0.86	63.2
951223.2	Bigger Guy	2.5	0.7	145.1	2.11	78.9
951221.2	Bigger Guy	2.5	1.0	142.0	1.04	61.9
951229.2	Bigger Guy	2.5	1.0	147.0	0.86	78.6
960425.1	Bigger Guy	3.2	1.0	139.7	1.37	87.5
960427.2	Bigger Guy	3.2	1.0	139.6	1.11	35.7
960422.1	Bigger Guy	3.2	1.0	144.0	0.46	30.8
951220.1	Long Tail	4.4	0.4	136.9	1.12	53.8
951222.1	Long Tail	4.4	0.4	137.1	0.90	40.0
960412.2	Long Tail	4.9	0.4	135.6	0.71	43.3
960415.1	Long Tail	4.9	0.4	135.6	1.13	61.1
951230.1	Long Tail	4.4	0.7	140.4	2.29	79.2
960101.1	Long Tail	4.4	0.7	138.3	0.65	59.1
951226.1	Long Tail	4.4	1.0	139.8	1.32	66.7
951228.1	Long Tail	4.4	1.0	139.8	0.81	73.9
960420.1	Long Tail	4.9	1.0	142.0	0.65	68.8
960422.2	Long Tail	4.9	1.0	143.4	0.90	68.4
960425.2	Long Tail	4.9	1.0	140.6	0.82	50.0
960428.1	Long Tail	4.9	1.0	144.9	1.60	41.2
951218.1	Small Sub	6.6	0.4	132.8	0.57	61.9
951220.2	Small Sub	6.6	0.4	135.6	1.01	66.7
960413.1	Small Sub	6.3	0.4	143.7	0.40	54.2
951222.2	Small Sub	6.6	0.7	136.9	0.61	50.0
951226.2	Small Sub	6.6	0.7	139.3	1.26	45.0
951228.2	Small Sub	6.6	1.0	137.6	1.00	30.0
951230.2	Small Sub	6.6	1.0	142.1	0.65	25.0
960420.2	Small Sub	6.3	1.0	134.1	10.00	84.0
960426.1	Small Sub	6.3	1.0	143.3	1.03	61.1
960428.2	Small Sub	6.3	1.0	141.3	0.92	85.7
951219.2	Big Sub	12.0	0.4	130.8	1.69	68.2
951227.1	Big Sub	12.0	0.4	138.6	0.45	61.8
960413.2	Big Sub	13.5	0.4	141.1	0.86	60.0
960416.1	Big Sub	13.5	0.4	143.2	0.60	71.4
951221.1	Big Sub	12.0	0.7	136.7	0.36	63.2
951229.1	Big Sub	12.0	0.7	130.9	1.89	61.3
951223.1	Big Sub	12.0	1.0	131.2	0.61	67.9
951231.1	Big Sub	12.0	1.0	137.1	0.66	61.9
960421.1	Big Sub	13.5	1.0	140.3	0.92	46.7
960424.1	Big Sub	13.5	1.0	135.0	1.40	55.0
960426.2	Big Sub	13.5	1.0	141.2	0.62	40.0
960414.1	B-1	18.4	0.4	141.1	0.63	50.0
960421.2	B-1	18.4	1.0	139.5	2.85	71.4
960424.2	B-1	18.4	1.0	144.5	0.74	28.6
960427.1	B-1	18.4	1.0	139.2	1.66	42.9

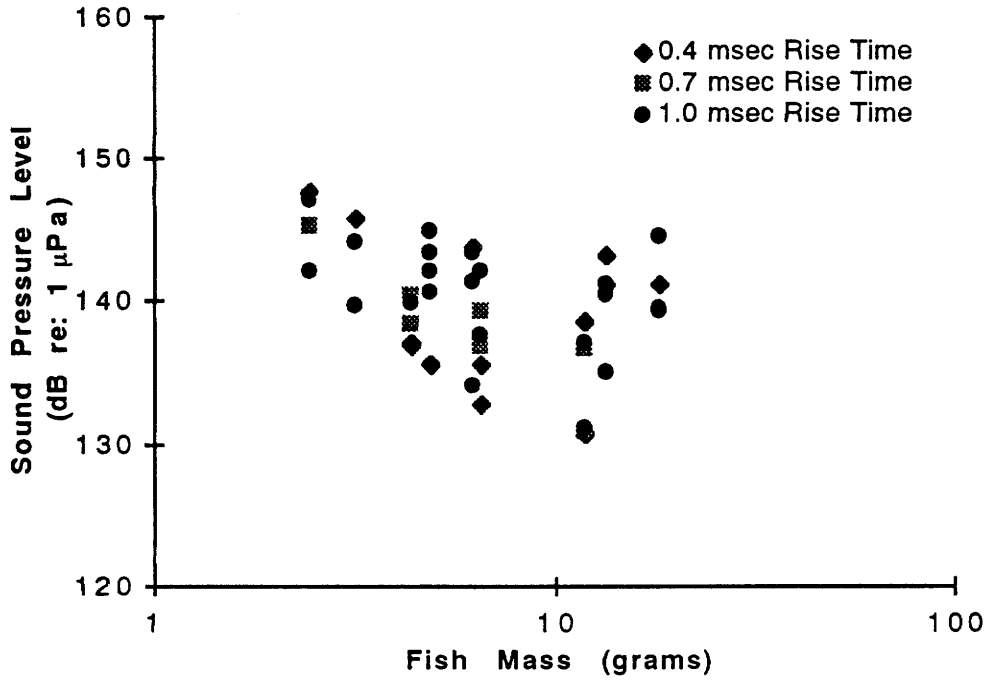

Figure 11. Sound pressure level at threshold as a function of fish mass.

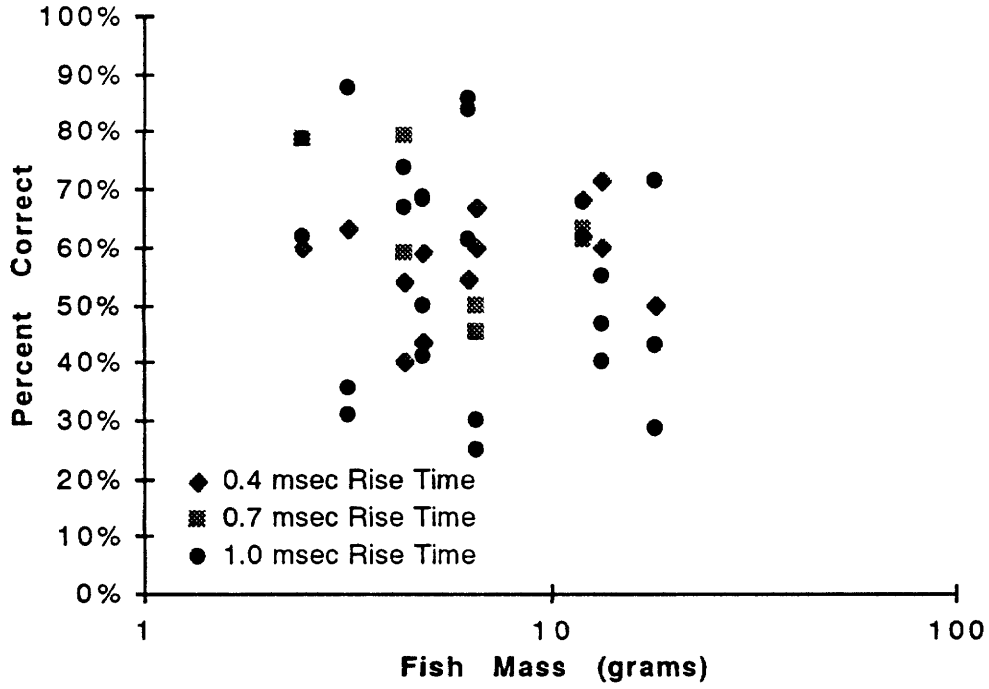

Figure 12. Percent correct response as a function of fish mass.

This is an interesting and unexpected trend: the fish doesn't always initiate the startle response by turning away from the source. Actually, the fish is more likely to turn in a random direction (50% correct / 50% incorrect). Investigating this lead to another conclusion: the acoustic pressure isn't the relevant acoustic parameter which initiates the startle.

The next step was to collect all of the data from the individual trials and divide it into bins based on range from the subject to the acoustic source. Figure 13 shows that, beyond a range of about 18 in. (46 cm.), the fish responded in a random direction (50% correct). But as the fish approached the source, it was more likely to turn away from the sound source.

Pressure threshold was also calculated versus range by dividing the data into four range bins and curve fitting the Weibull function to each bin. The results, shown in Figure 14, indicate that the acoustic pressure level at threshold rises as the subject gets further from the source. If acoustic pressure alone initiated the startle response, then the threshold level should be independent of range.

Since the otolithic organs of the fish's ear are thought to work like accelerometers, the data was reexamined in terms of acoustic acceleration.

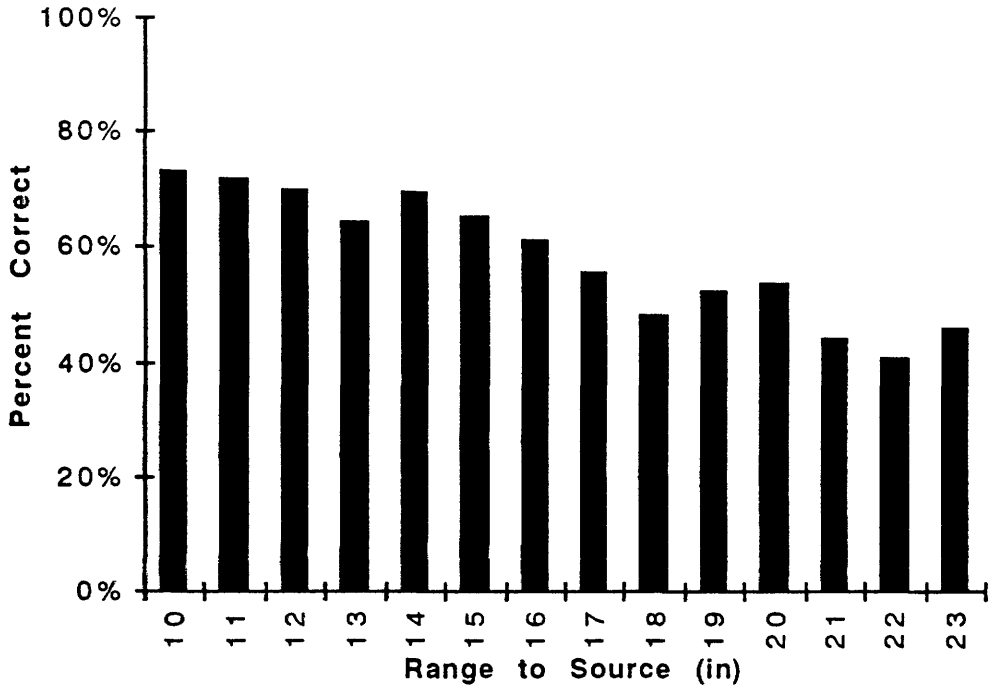

Figure 13. Percent correct response as a function of distance from the source.

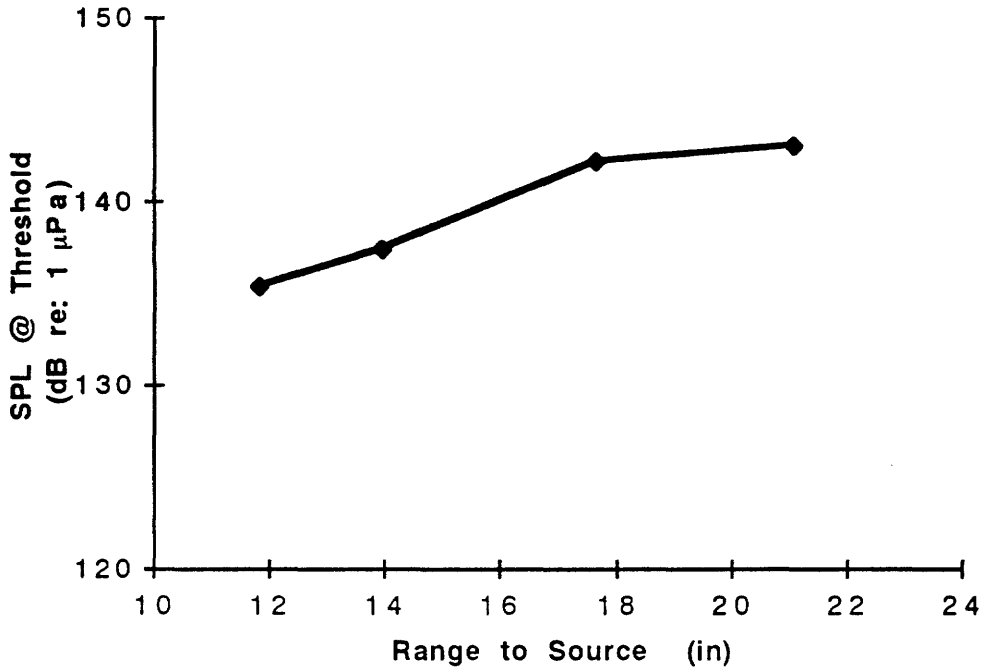

Figure 14. Sound pressure level at threshold as a function of distance from the source.

The acoustic acceleration, a, can be calculated from the measured pressure signal, p, by the relation

$$a = \frac{1}{\rho c}\frac{\partial p}{\partial t} + \frac{p}{\rho r} \qquad (2)$$

where ρc are the density and sound speed of the water, and r is the range to the source [Pierce, 1981]. Note that the second term has an additional 1/r dependence. Therefore, unlike the pressure signal, the acceleration signal changes shape as it propagates away from the source. For the signals used in this study, however, the rapid pressure rise causes the first term to dominate the second initially, and the signal changes shape with range only later in the pulse [Fig. 15]. The rise times for the acceleration signals were not significantly different than the pressure signals. Figure 16 shows the temporal relation for acoustic pressure and acoustic particle motion; only the acceleration is relatively in-phase for the transient stimulus used. The data from individual trial series were fit to a Weibull function, and the results are given in Table 2 and Figure 17.

Again, the data was separated into bins by range and thresholds calculated [Fig. 18]. Although a linear regression of the data indicates a -2 dB per octave slope in threshold with

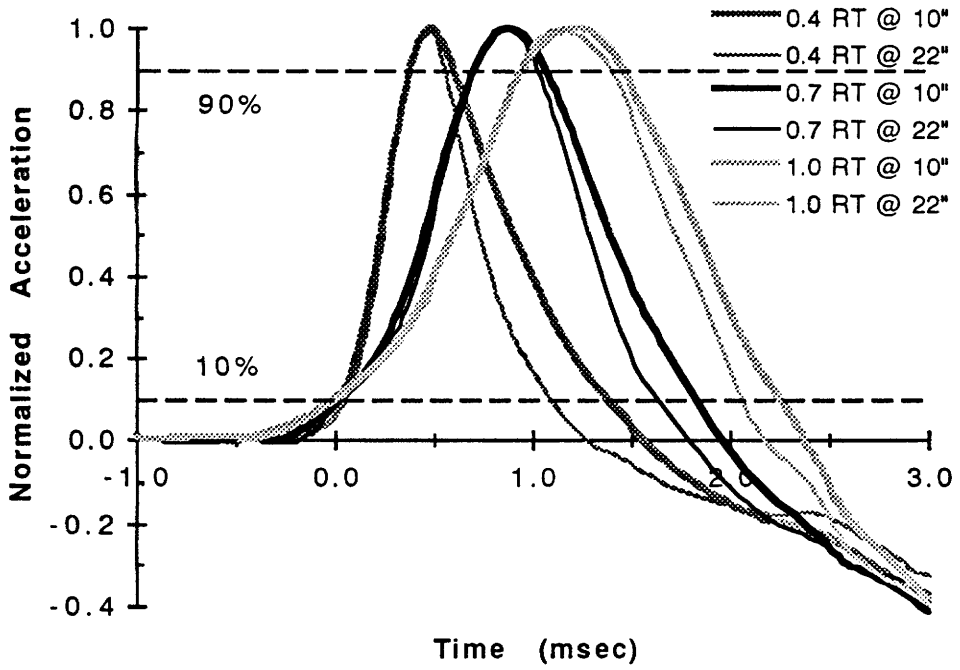

Figure 15. Determination of rise time for the three acceleration signals. The thick lines are at 11" range. The corresponding thin line is the same signal after propagating an additional 12". Each curve has been normalized to peak acceleration in amplitude and aligned at 10% peak in time.

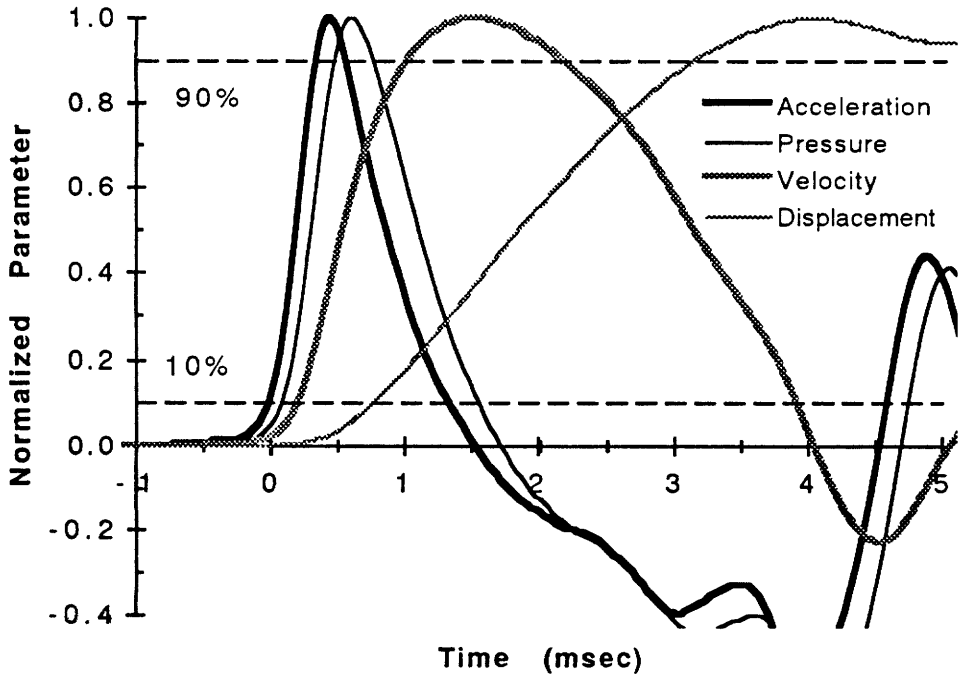

Figure 16. Measured acoustic pressure and calculated acoustic acceleration, velocity and displacement for a stimulus signal.

Table 2. Curve fit for the acoustic acceleration threshold series to the Weibull function.

Date (yymmdd.n)	Fish Name	Weight (g)	Rise Time (msec)	Accel. Threshold (dB)	Slope (β)	% Correct
951219.3	Bigger Guy	2.5	0.4	-24.0	0.69	60.0
960414.2	Bigger Guy	3.2	0.4	-27.4	10.00	66.7
951223.2	Bigger Guy	2.5	0.7	-28.6	0.97	78.9
951221.2	Bigger Guy	2.5	1.0	-32.1	0.97	61.9
951229.2	Bigger Guy	2.5	1.0	-27.1	1.13	78.6
960425.1	Bigger Guy	3.2	1.0	-29.9	1.07	87.5
960427.2	Bigger Guy	3.2	1.0	-29.5	0.68	35.7
960422.1	Bigger Guy	3.2	1.0	-30.2	0.83	30.8
951220.1	Long Tail	4.4	0.4	-33.1	2.16	53.8
951222.1	Long Tail	4.4	0.4	-34.0	1.10	40.0
960412.2	Long Tail	4.9	0.4	-30.0	0.51	43.3
960415.1	Long Tail	4.9	0.4	-27.8	1.06	61.1
951230.1	Long Tail	4.4	0.7	-28.1	1.64	79.2
960101.1	Long Tail	4.4	0.7	-33.4	0.74	59.1
951226.1	Long Tail	4.4	1.0	-31.6	0.89	66.7
951228.1	Long Tail	4.4	1.0	-30.5	1.09	73.9
960420.1	Long Tail	4.9	1.0	-26.9	0.68	68.8
960422.2	Long Tail	4.9	1.0	-28.1	0.81	68.4
960425.2	Long Tail	4.9	1.0	-27.8	10.00	50.0
960428.1	Long Tail	4.9	1.0	-27.3	1.00	41.2
951218.1	Small Sub	6.6	0.4	-34.7	0.82	61.9
951220.2	Small Sub	6.6	0.4	-31.1	1.07	66.7
960413.1	Small Sub	6.3	0.4	-28.3	0.47	54.2
951222.2	Small Sub	6.6	0.7	-36.1	2.08	50.0
951226.2	Small Sub	6.6	0.7	-35.5	1.22	45.0
951228.2	Small Sub	6.6	1.0	-35.2	3.58	30.0
951230.2	Small Sub	6.6	1.0	-33.2	1.49	25.0
960420.2	Small Sub	6.3	1.0	-35.3	1.47	84.0
960426.1	Small Sub	6.3	1.0	-25.6	1.19	61.1
960428.2	Small Sub	6.3	1.0	-28.8	0.98	85.7
951219.2	Big Sub	12.0	0.4	-36.2	10.00	68.2
951227.1	Big Sub	12.0	0.4	-31.0	1.72	61.8
960413.2	Big Sub	13.5	0.4	-31.5	1.28	60.0
960416.1	Big Sub	13.5	0.4	-23.1	0.65	71.4
951221.1	Big Sub	12.0	0.7	-36.8	10.00	63.2
951229.1	Big Sub	12.0	0.7	-35.0	1.42	61.3
951223.1	Big Sub	12.0	1.0	-38.0	2.16	67.9
951231.1	Big Sub	12.0	1.0	-33.4	1.42	61.9
960421.1	Big Sub	13.5	1.0	-33.9	3.69	46.7
960424.1	Big Sub	13.5	1.0	-34.7	1.03	55.0
960426.2	Big Sub	13.5	1.0	-32.7	5.36	40.0
960414.1	B-1	18.4	0.4	-28.2	0.39	50.0
960421.2	B-1	18.4	1.0	-28.6	0.76	71.4
960424.2	B-1	18.4	1.0	-22.3	0.78	28.6
960427.1	B-1	18.4	1.0	-26.4	1.81	42.9

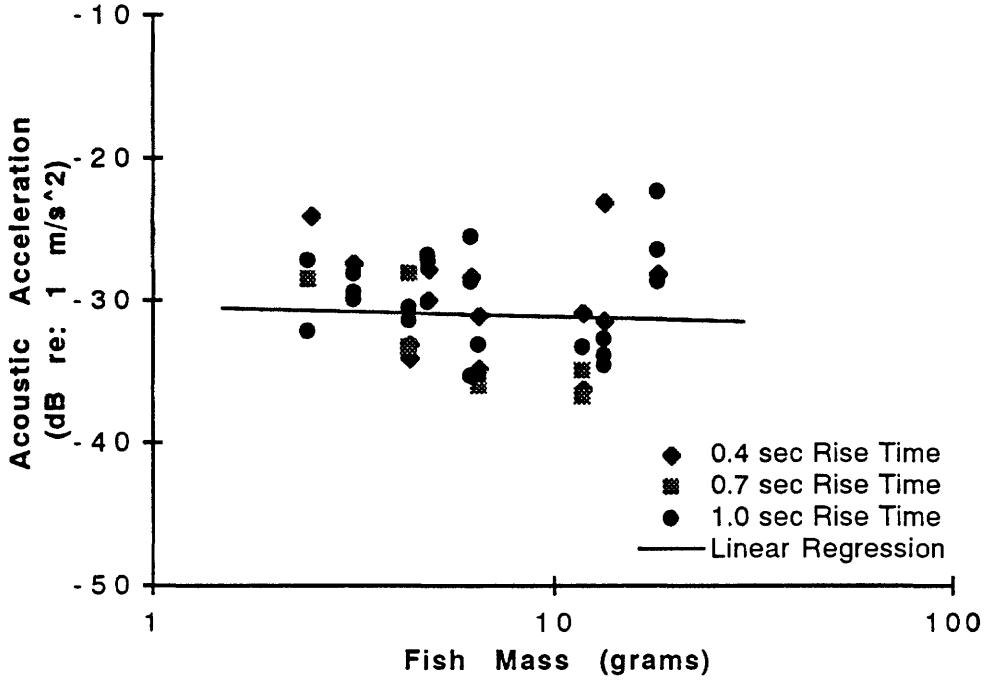

Figure 17. Threshold acoustic acceleration level for each series as a function of fish mass. The linear regression to the data is a = −30.44 − 0.037 * m (dB).

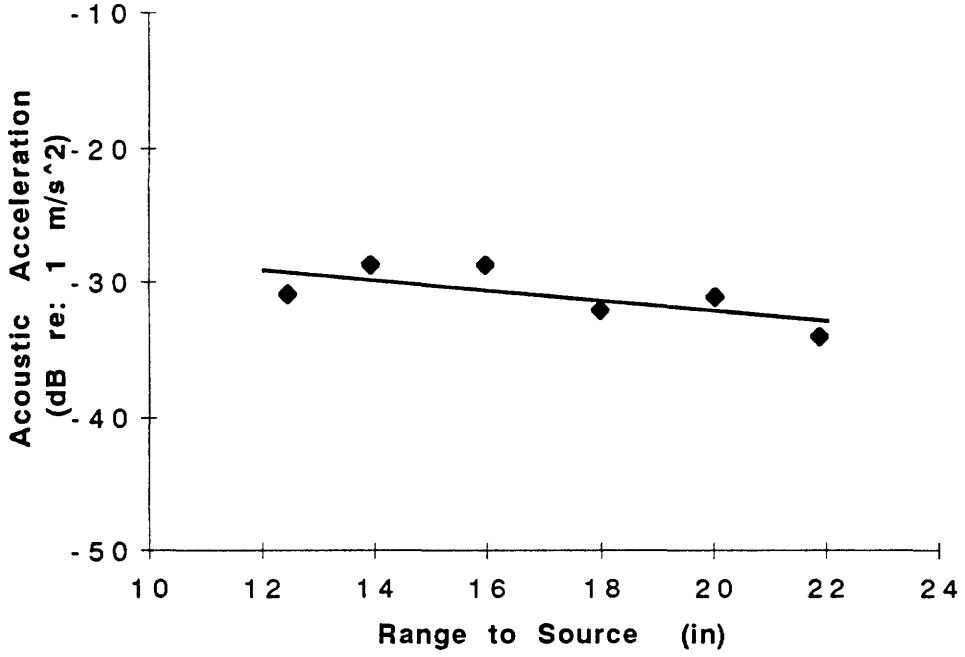

Figure 18. Peak acoustic acceleration at threshold as a function of range to the source. The data points fall within a range of 5 dB, which was minimum stimulus step size used. The linear regression to the data is a = −24.50 − 0.37 * r (dB).

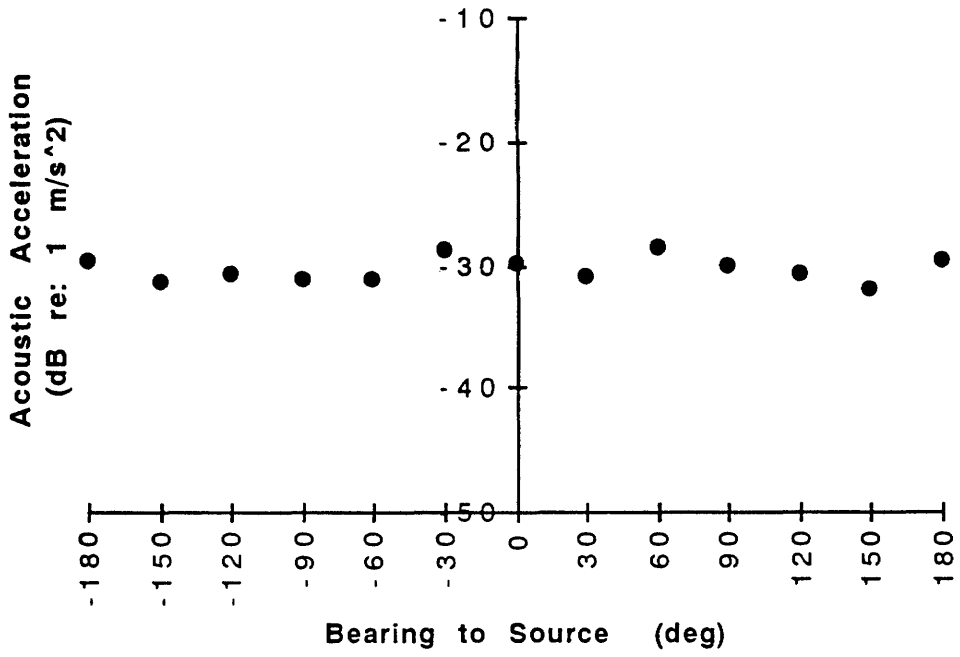

Figure 19. Peak acoustic acceleration at threshold as a function of bearing to the source. The data from all trials were collected in bearing bins 30° wide centered on the bearings shown and fit to Weibull functions.

range, the data are within 5 dB, which was the stimulus step size used to determine thresholds. The data was also separated into bins by bearing angle and thresholds calculated [Fig. 19]. The thresholds ranged within 3 dB, so there is no measured functional dependence.

One last look at the data is necessary to understand the underperformance of the subjects in choosing the correct direction to respond. First, the trials where the subject startled were collected. The calculated acceleration levels were then normalized with the threshold levels for that trial series. The data was then sorted in bins based on the relative acceleration level. The diamonds in Figure 20 represent correct percentages for the bins. For all data points with relative acceleration below zero dB, the percent correct was 51%. Although the data point at -10 dB may indicate a linearly decreasing trend, this bin contains only 12 of the 154 sub-zero trials. Above zero, the percent correct increases with relative signal level. The linear regression illustrated begins at 50% correct for 0 dB and increases at a slope of 1.4 %/dB.

By our measure, the directional startle response is binary; the fish either turns to the left or to the right. Therefore, the lateral component of the acceleration signal may be more

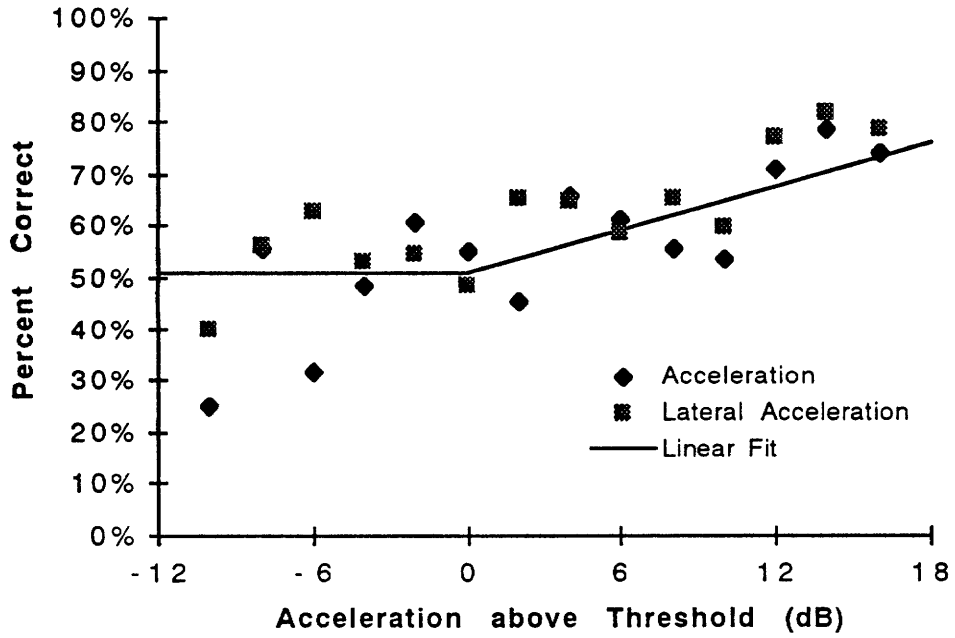

Figure 20. Percent correct directional response as a function of the relative magnitude of the acceleration level for each response trial to the calculated threshold level for that series.

important in determining direction of response than the fore-aft component. To test this, the correctness was examined relative to the peak lateral acceleration. The magnitude of the lateral acceleration for all of the individual trials were calculated by multiplying the peak acceleration by the absolute value of the sine of the bearing angle. The lateral acceleration was normalized with the threshold peak acceleration for that trial series and separated into bins by acceleration above threshold. The percent correct in each bin is shown as boxes in Figure 20.

The absolute value of the sine of the bearing angle varies from 0 for head-on or tail-on source to a maximum of 1 when the source is directly to the left or right. If correctness is determined only by the lateral acceleration, the effect of this operation is to filter random response trials (50% correct) to the left in Figure 20, raising the correctness of the remaining supra-threshold trials. On inspection, this appears to be true, as supra-threshold trials went from 60.7% to 64.0% correct, while the sub-threshold trials averaged 51.0% before and 53.2% after.

Within the limits of the experimental parameters, the results from this study can be summarized as follows:

- A startle response occurred when the acoustic acceleration reached a level of about -30 dB re: 1 m/s^2.
- The threshold was not dependent upon the rise time of the signal.
- The threshold was not dependent upon the bearing to the source.
- The fish was more likely to turn away from the sound source as the lateral acoustic acceleration exceeded the threshold level.

Discussion

In order to understand the startle system, a clear understanding of the function of otolithic endorgans of the ear is necessary. An otolithic organ consists of a dense calcareous otolith overlying a macula containing sensory hair cells [Fig. 21]. Between the otolith and the macula (but not shown in the figure) is a gelatinous membrane [Platt, 1977] that is thought to act as an elastic coupling [Rogers and Cox, 1988]. Individual hair cells are directional, in that bending the cilia toward the tallest cilia (the kinocilia) causes depolarization, bending away causes hyperpolarization, and bending off axis results in a cosine function response [Flock, 1971]. The adequate stimulus for eliciting this response in hair cells is displacement of the cilia. Typically, the hair cells in the macula are arranged into pairs of oppositely oriented groups [Platt, 1977].

Since a fish has about the same density as the surrounding water, it moves with the acoustic particle motion in a low frequency sound field [Lewis, 1994]. The macula, highly innervated from one side, probably follows along. The otolith, however, doesn't have to. The right side of Figure 21 shows a simple dynamic model of the otolithic organ. The large block on the left represents the body of the fish, which freely moves back and forth with the sound on the left represents the body of the fish, which freely moves back and forth with the sound field (the motion of the otolith doesn't affect the motion of the fish) with displacement y(t). The otolith is a small mass, m, and moves with displacement x(t). Connecting the two is a spring (representing the elastic membrane) with stiffness k. The relative motion between the two is $z(t) = x(t) - y(t)$. By examining a free-body diagram of the otolith, the equation of motion can be obtained:

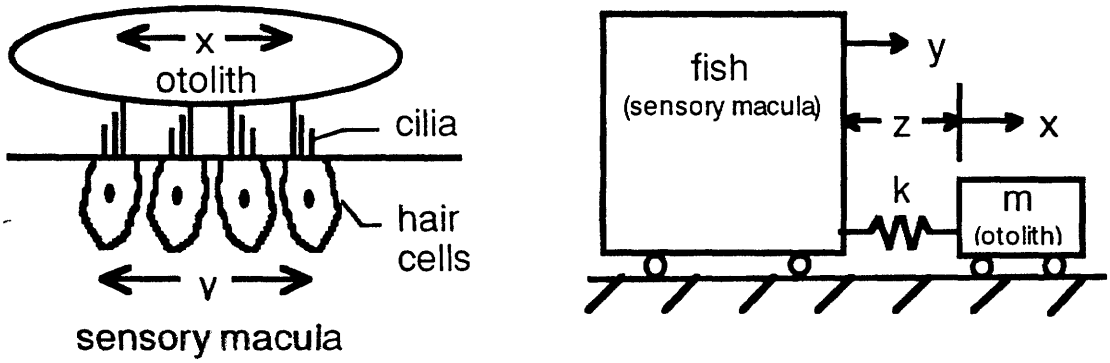

Figure 21. Pictorial representation and simple dynamical model of an otolithic organ in response to an acoustic wave.

$$m\ddot{z} + kz = -m\ddot{y}. \tag{3}$$

where each dot over a variable indicates a time derivative. Assuming a sinusoidal displacement of the fish $y = Y\sin(\omega t)$, where Y is the maximum amplitude and $\omega = 2\pi f$ the angular frequency, the amplitude of steady state solution is

$$Z = \frac{Y\omega^2}{\omega_o^2\left(1 - \frac{\omega^2}{\omega_o^2}\right)} \tag{4}$$

where Z is the maximum amplitude of the relative motion and $\omega_o = \sqrt{\frac{k}{m}}$ is the natural resonance frequency of the system [Rao, 1990]. Noting that $\ddot{y} = -Y\omega^2\sin(\omega t)$, so that when the otolith is being driven at a frequency below its natural frequency, $\omega << \omega_o$, the term in the parentheses of Equation 4 is approximately 1 and

$$Z = \frac{1}{\omega_o^2} Y\omega^2 \tag{5}$$

or the magnitude of the relative displacement is proportional to the magnitude of the acceleration of the fish. Since relative displacement between the otolith and the macula bends the cilia of the hair cells, acoustic acceleration is an appropriate stimulus for the otolithic organ. This type of analysis has been supported by experimentation, as displacement detection thresholds for direct head vibrations in goldfish decrease with ω^2 [Fay and Patricoski, 1980]. This is referred to in the literature as the "direct" method of inner ear stimulation.

But detection of the acoustic particle motion alone isn't enough to directionalize a simple source. Identical sources with inverted signals 180° apart will produce the same stimulation of the otoliths. This ambiguity can be resolved by knowing the relative phase of the acoustic pressure to the acoustic acceleration [Schuijf and Buwalda, 1975]. In the near field, the direction of the acceleration points away from the source when the pressure is at its maximum [Fig. 16, for example]. Therefore, pressure detection is required for directionalization. In order for an otolithic organ to detect pressure, there must be a transformation of acoustic pressure into something it does detect.

Many fish have air-filled swimbladders which effectively scatter sound underwater [Sand and Hawkins, 1974; Lewis, 1994]. Positive acoustic pressure compresses the

swimbladder and negative pressure expands it. This vibrating swimbladder itself acts like a simple source, radiating sound. Because the resonance frequency of the swimbladder is usually above the hearing range of the fish, the swimbladder acts like a spring - displacement of the swimbladder wall is proportional to the incident pressure [Rogers et al., 1988]. Since acceleration is two time derivatives of displacement, the amplitude of the radiated acoustic acceleration is proportional to two time derivatives of the incident acoustic pressure. For fish with simple swimbladders, the otolithic organ responds to the second time derivative of the acoustic pressure, $\ddot{p}$. The validity of this "indirect" path has been demonstrated by measuring changes in acoustic detection thresholds between inflated and deflated swimbladders [Sand and Enger, 1973].

Hearing specialists have enhanced couplings between the swimbladder and their ear. Fish of the superorder Otophysi, including goldfish, have Weberian ossicles which act as part of a mechanical/hydraulic system which couples the motion of the swimbladder to the ear [Dijkgraaf, 1960]. The ossicles are a series of small bones connected by ligaments and articulate around nearby vertebrae [Fig. 22]. The most posterior ossicle is attached to the tunica externa of the swimbladder. The most anterior ossicle, the scaphium, is incorporated into the wall of a central fluid-filled cavity, the sinus impar. The saccule of the two ears are connected by a transverse canal. On expansion of the swimbladder, the ossicles move forward, the scaphia press on the sinus impar driving fluid in it forward, compressing the posterior diverticulum of the transverse canal, and displacing fluid from it into the sacculi [Alexander, 1966].

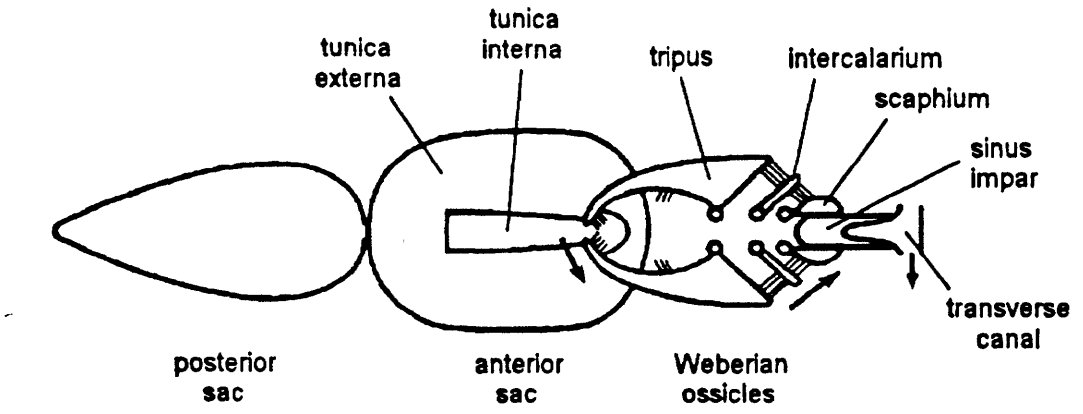

Figure 22. Diagram showing the function of the swimbladder and Weberian ossicles of the goldfish. Arrows indicate the direction of motion due to the rarefaction phase of an acoustic wave. (From Alexander, 1966.)

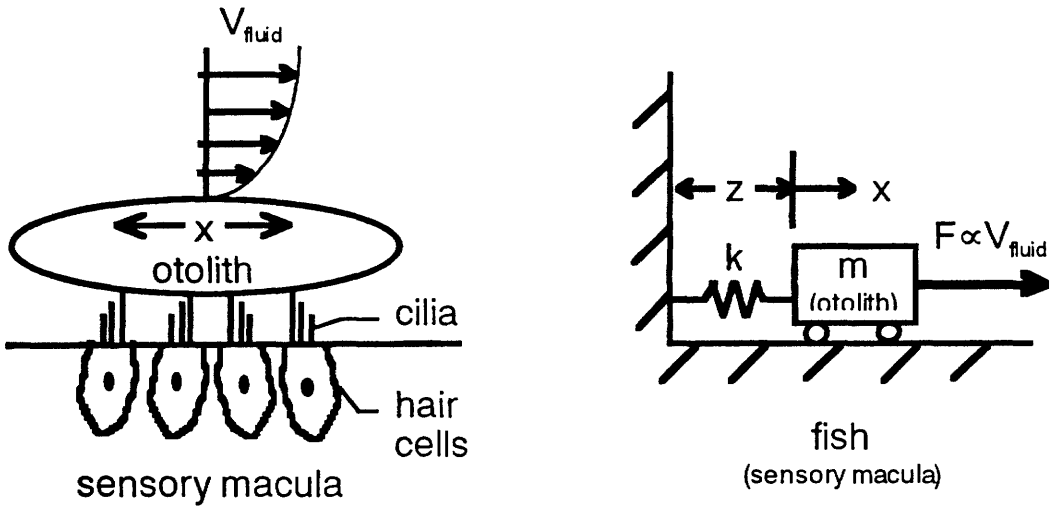

Figure 23. Pictorial representation and simple dynamical model of an otolithic organ in response fluid flow over the otolith. The fluid oscillates over the otolith with the acoustic pressure by way of the Weberian ossicles.

The displaced fluid flowing in the saccular chamber could be responsible for the stimulation of the ear by viscous action. The viscous force on the otolith from fluid passing over it is proportional to the velocity of the fluid. The flow provides an oscillatory force which moves the otolith over the stationary macula. For the simple dynamical model shown in Figure 23, the equation of motion is

$$m\ddot{z} + kz = F\cos\omega t. \tag{6}$$

where F is the magnitude of the force applied to the otolith, and the solution is

$$Z = \frac{F}{\left(1 - \frac{\omega^2}{\omega_o^2}\right)}. \tag{7}$$

Below resonance, the term in parenthesis goes to 1 so the relative displacement of the otolith to the macula is proportional to the force, which is proportional to the fluid velocity. As before, data on vibrational sensitivity suggests that the resonance frequency of the otolithic organs is above the hearing range.

Since acoustic pressure displaces the swimbladder and the Weberian ossicles transfers this displacement to the fluid in the saccular sac, the time derivative of the acoustic pressure is proportional to the fluid velocity around the saccular otolith. The force on the otolith is

proportional to the fluid velocity, and the magnitude of the force is proportional to the displacement of the otolith. Therefore, since relative displacement between the otolith and the macula bends the cilia of the hair cells, the time derivative of acoustic pressure, $\dot{p}$, is an appropriate stimulus for the otolithic organs connected to the Weberian ossicles.

Therefore, there are three potential mechanisms for acoustic waves to stimulate the ear. First, the acoustic acceleration can directly stimulate an otolithic organ. Second, for fish with swimbladders, acoustic pressure can be reradiated from the swimbladder and the stimulation is proportional to $\ddot{p}$. Third, for fish with Weberian ossicles, the motion of compression of the swimbladder by acoustic pressure is transmitted to the saccular sac and the stimulation is proportional to $\dot{p}$.

Individual auditory and vestibular nerve fibers in the goldfish are directionally sensitive to whole body acceleration [Fay, 1984]. A cell's threshold for 140 Hz motion was determined for each axis through the use of a tracking procedure with a phase locking criterion. The two-dimensional directivity of individual hair cells is transformed into three-dimensional directivity in the nerve fibers of the eighth nerve which innervate the ear. Best thresholds to linear acceleration vary as a function of the direction of motion according to a three-dimensional dipolar function.

Each otolithic organ has a characteristic directional response pattern [Fay, 1984]. Neurons in the saccule have similar best response directions which point along a common axis. In the utricle, however, fibers respond maximally at a variety of azimuths in the horizontal plane. The fibers of the lagena also cluster on a plane, but that plane is vertically oriented.

Fay and Ream [1986] surveyed the physiological characteristics of goldfish saccular fibers stimulated by underwater sound and classified them into four types. In contrast to the first three types, the fourth fiber type showed no frequency selectivity, had high thresholds, and tended to respond with a phasic or rapidly adapting response. This type performs similarly to the large diameter fibers classified as S1 by Sento and Furukawa [1987]. These are the ideal nerve fibers for providing input to the Mauthner cells [Fay, 1995]. They also do not appear to be represented at the level of the midbrain [Lu and Fay, 1993].

The specific end organs of the ear projecting to the Mauthner cell are not known [Popper and Edds-Walton, 1995]. Although most sensory input influences the Mauthner cell indirectly through interneurons [Zottoli et al., 1995], large diameter axons in the eighth nerve have been found which project to the lateral dendrite of the Mauthner cell [Lin et al., 1983].

Large diameter fibers have been identified in the goldfish innervating the macula of the saccule [Sento and Furukawa, 1987] and leaving the lagena and the utricle [Popper and Edds-Walton, 1995]. Zottoli et al. [1995] also found that fibers from the utricular portion of the VIIIth nerve terminate on the distal lateral dendrite of the Mauthner cell. In the goldfish, subthreshold stimulation of the anterior branch of the eighth nerve (innervating the utricle) caused a small post-synaptic potential in the Mauthner cell. In combination with subthreshold input to the posterior branch of the eighth nerve (innervating the saccule and lagena), stimulation of the anterior branch was sufficient to reach threshold [Zottoli and Faber, 1979].

The initial proposal for this research project described a model for the directional acoustic startle response. The goal was to use anatomical and physiological information on the ear and the Mauthner cell to link the functional directionality of the escape response with the acoustic stimulation which elicits it. Suprisingly, a similar model was proposed and published by another research group [Eaton et al., 1995]. The original model has been revised based of the results of this study and is presented below.

The revised model was based on the following assumptions:

- The prey startles when the predator accelerating toward the prey (generating a positive pressure pulse with acoustic acceleration directed away from the source) or when the predator tries to suck the prey into its mouth (generating a negative pressure pulse with acoustic acceleration directed toward the source).
- The fish responds to the initial pulse of the acoustic wave.
- The fish detects both the initial acceleration pulse and the initial pressure pulse, startling after both exceed threshold values. (Although the model is described in terms of pressure, the analysis above indicates that the goldfish's ear may be more sensitive to $\dot{p}$ or $\ddot{p}$. But their amplitudes are proportional to pressure, so the model will use pressure as a surrogate and the implications will be discussed later.)
- The response is ballistic. Once the fish starts moving, no additional information is collected.

Figure 24 shows the revised model. The system uses two paired acoustic detectors. The utricles directly detects the acoustic acceleration and the saccules indirectly detects the acoustic pressure either through the scattering of sound by the swimbladder or through the motion of the Weberian ossicles. The utricle was chosen over the lagena or saccule for

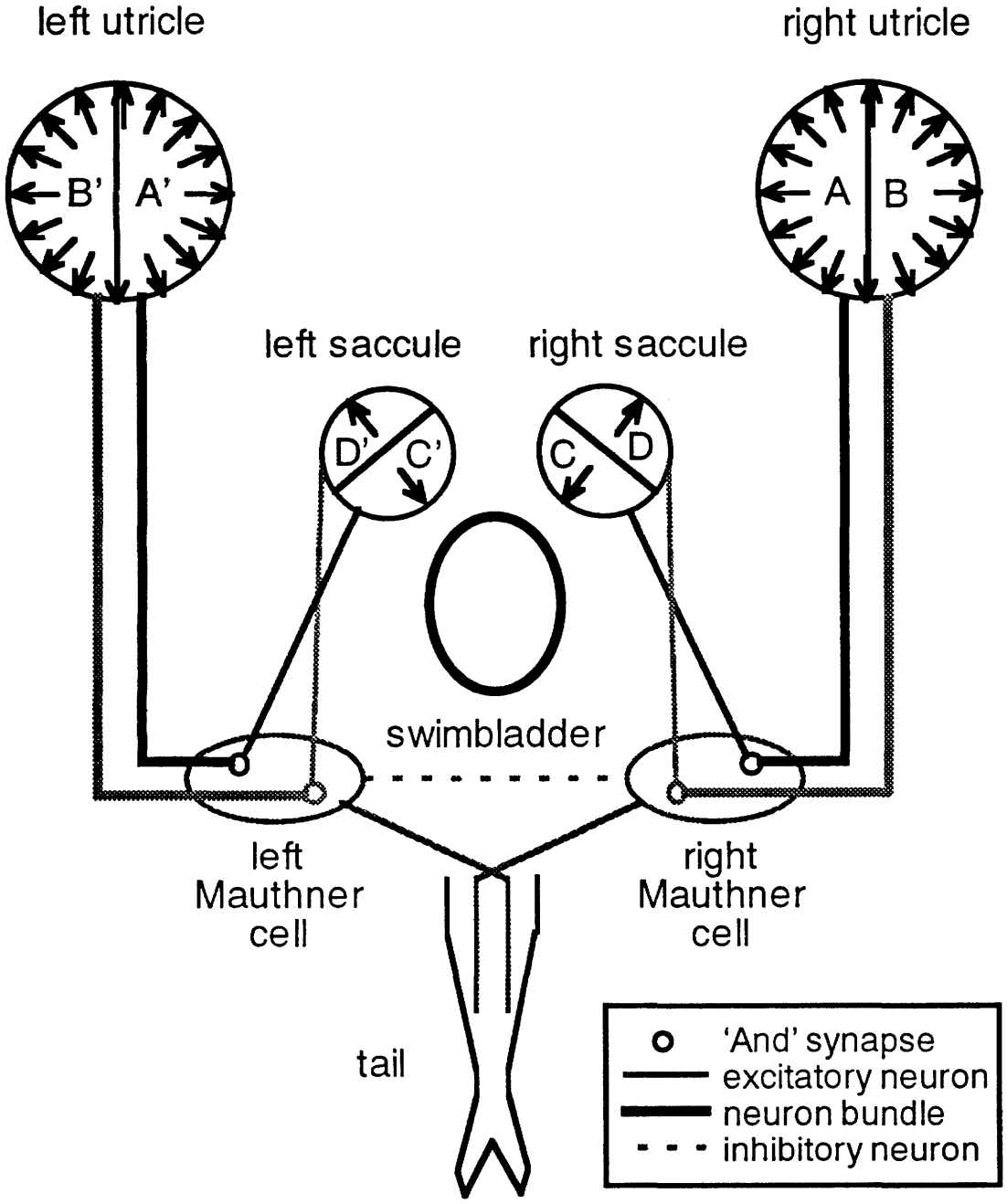

Figure 24. Model for the directional startle reflex in the goldfish.

acceleration detection because utricular nerve fibers responded best in the horizontal plane over a wide range of azimuths [Fay, 1984]. Including lagenar to give two-dimensional (azmuthal and elevational) sensitivity is conceptually trivial, but the data to test this is not yet available. The arrows in Figure 24 indicate the directional sensitivity of the nerve fibers in each endorgan.

The nerve fibers in the neuron bundle primarily responsible for the startle response can be characterized as having no spontaneous activity and poor sensitivity. These characteristics imply the large fibers described earlier.

The nerve fibers for each endorgan are bundled into two groups based on their directivity. For the utricle, medial directed fibers are bundled (A and A') separate from lateral directed fibers (B and B'). For the saccule, fibers that are responsive to motion directed toward the swimbladder (C and C') are segregated from fibers responsive to motion away (D and D'). The bundles are connected to their ipsilateral Mauthner cell in specific pairs, with one from each endorgan. Between the two Mauthner cells is an inhibitory neuron so that firing one prevents the other from firing. Note that the axons of the Mauthner cells cross; the left Mauthner cell causes contraction of the musculature on the right side of the fish, turning it to the right.

The physics of the model is simple. Positive acoustic acceleration directed toward the left stimulates fiber bundles A and B' and to the right A' and B. Positive acoustic pressure causes the swimbladder to shrink, pulling the fluid around the saccule toward the swimbladder, exciting fiber groups C and C'. Negative pressure expands the swimbladder, exciting D and D'.

Two pairs of nerve bundles synapse onto each Mauthner cell and function as logical And gates. If fibers from both the utricle (acceleration) and saccule (pressure) fire on the same synapse, then that Mauthner cell fires and the other is inhibited. Table 3 shows the response of the model to the two different prey capture techniques (accelerating toward the prey and sucking it in) on either side. A predator on the right accelerating toward the prey launches a positive pressure wave with the initial acceleration to the left. This excites fibers in bundles C, C', A, and B'. Since A and C both excite the same synapse, the right Mauthner cell fires and the fish turns to the left. If the predator on the right attempted to capture the prey using suction, the acoustic pressure wave would be negative and initial acceleration to the left, exciting D, D', A', and B. Only B and D stimulate the same synapse, so again, the right Mauthner cell is stimulated and the prey turns toward the left. For the predator on the right, either accelerating toward the prey or attempting suction capture causes the prey to respond by turning to the left. Conversely, the predator on the left causes the left Mauthner cell to fire and the prey turns away to the right. This combination of And gates is functionally identical to the Xnor gate described in Eaton et al. [1995]. They hypothesizes that this gating was implemented using inhibitory PHP neurons.

Table 3. Response of startle model to acoustic stimuli.

Location of Source	Action of Source	Direction of Lateral Acceleration	Sign of Acoustic Pressure	Excited Nerve Fiber Bundles	Effective 'And' Synapse	Excited Mauthner Cell
Right	Accelerate	Left	Plus	A B' C C'	A C	Right
Right	Suction	Right	Minus	B A' D D'	B D	Right
Left	Accelerate	Right	Plus	B A' C C'	A' C'	Left
Left	Suction	Left	Minus	A B' D D'	B' D'	Left

The principal revision to the model from the original is the inclusion of all directional fibers from the acceleration detector (utricle). The original model used only lateral acceleration detectors, predicting that neither Mauthner cell would be excited by head-on or tail-on stimuli and implying that threshold would be inversely proportional to the sine of the bearing angle to the source. The results from this study showed, however, that the startle threshold was independent of bearing angle. The alternative hypothesis that only acoustic pressure was necessary to trigger startles (thereby being omnidirectional) was rejected when the threshold level was found to be range dependent.

One advantage of this detection system is that it requires very little neural circuitry for effective performance. Figure 24 shows 16 independent directional sensors on each utricle and 2 on each saccule for a total of 36. With this array, the sensors are oriented 22.5° apart. The worst case threat in the horizontal plane would come from a source whose bearing bisected adjacent detectors. The difference in response threshold for this threat compared to the optimal performance of the system (predator aligned with a detector) is only 0.2 dB. Cutting the number of evenly spaced sensors on the utricle in half would only increase the worst case detection threshold by 0.7 dB.

Not only can the model explain the correct responses, it may be useful in understanding incorrect responses. Figure 20 shows that as the acoustic acceleration relative to threshold is reduced, the directionality of the fish becomes more random. This performance can be understood in the context of the model by looking at the timing of the information to the Mauthner cell from the ear. Either Mauthner cell fires when acoustic pressure and acceleration information both stimulate the appropriate channel at the same time.

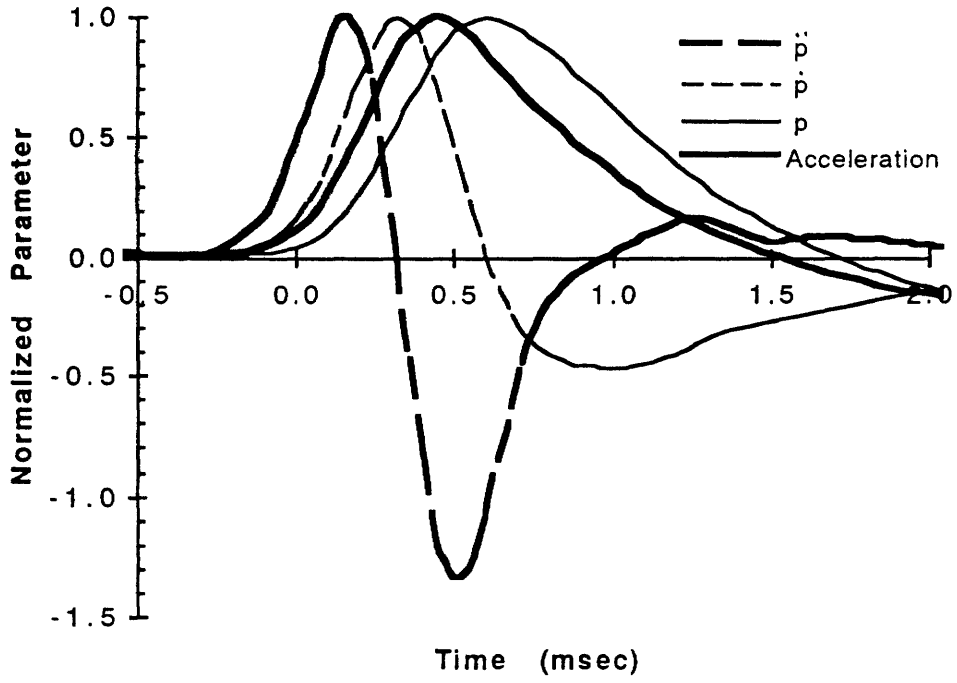

Figure 25. Acoustic pressure, acceleration, and first and second time derivatives of acoustic pressure $\dot{p}$ and $\ddot{p}$ for the 0.4 msec rise time signal.

Earlier was a discussion on the mechanisms by which acoustic pressure stimulates the otolithic organs. For fish with swimbladders, $\ddot{p}$ was considered the relevant parameter characterizing the stimulation, and for fish with Weberian ossicles, $\dot{p}$ was hypothesized. Figure 25 shows the acceleration and pressure along with $\dot{p}$ and $\ddot{p}$ for the acoustic signals used in this study.

The key to understanding the randomness of the near threshold responses is that the relevant pressure parameters, $\dot{p}$ or $\ddot{p}$, can be either positive or negative when the acceleration channel is triggered. Since the measured thresholds were in acceleration, the pressure channel is well above threshold and gives accurate and timely information of its acoustic variable. As shown in Figure 25, however, both $\dot{p}$ and $\ddot{p}$ quickly change sign. The initial positive pulse of the acoustic acceleration is longer in duration. Near threshold, the acceleration channel fires at some time during the interval that the acceleration is near its peak. So the direction of the response also depends on when the acceleration channel fires, as the pressure channel may have already passed through the change in its sign. As the acoustic signals further exceeds threshold, the acceleration channel triggers earlier, so it becomes more likely that the corresponding pressure channel will have the proper sign. Thus well above threshold, the

correctness of responses will approach 100 percent. At threshold, when the trigger occurs near the peak of the acceleration channel signal, the correctess will be close to 50 percent. The exact timing shown in Figure 25 only applies for the acoustic signal, as the time delays in the sensory response and neural transmission for each endorgan are not known.

Bibliography

Alexander, R.McN. (**1966**). "The physical aspects of swimbladder function," Biol. Rev. **41**, 141-172.

Canfield, J.G. and R.C. Eaton (**1990**). "Swimbladder acoustic pressure transduction initiates Mauthner -mediated escape," Nature **347**, 760-762.

Dijkgraaf, S. (**1960**). "Hearing in bony fishes," Proc. Roy. Soc. Lond. Ser. B. **152**, 51-54.

Eaton, R.C., R.A. Bombardieri, and D.L. Meyer (**1977**). "The Mauthner-initiated startle response in teleost fish," J. Exp. Biol. **66**, 65-81.

Eaton, R.C., J.G. Canfield, and A.L. Guzik (**1995**). "Left-right discrimination of sound onset by the Mauthner system," Brain Behav. Evol. **46**, 165-179.

Eaton, R.C. and D.S. Emberly (**1991**). "How stimulus direction determines the trajectory of the Mauthner-initiated escape response in a teleost fish," J. Exp. Biol. **161**, 469-487.

Eaton, R.C. and A.N. Popper (**1995**). "The octavolateralis system and the Mauthner cell: Interactions and questions," Brain Behav. Evol. **46**, 124-130.

Fay, R.R. (**1984**). "The goldfish ear codes the axis of acoustic particle motion in three dimensions," Science **225**, 951-954.

Fay, R.R. (**1995**). "Physiology of primary saccular afferents of goldfish: Implications for Mauthner cell response," Brain Behav. Evol. **46**, 141-150.

Fay, R.R. and S. Coombs (**1992**). "Psychometric functions for level discrimination and the effects of signal duration in the goldfish (*Carassius auratus*): Psychophysics and neurophysics," J. Acoust. Soc. Am. **92**, 189-201.

Fay, R.R. and M.L. Patricoski (**1980**). "Sensory mechanisms of low frequency vibration detection in fishes," in *Abnormal Animal Behavior Proceeding Earthquakes, Conference II*, edited by R. Burskirk, U.S. Geological Survey Open File Report, 80-453.

Fay, R.R. and T.J. Ream (**1986**). "Acoustic response and tuning in saccular nerve fibers of the goldfish, (*Carassius auratus*)," J. Acoust. Soc. Am. **79**, 1883-1895.

Flock, A. (**1971**). "Sensory transduction in hair cells," in *Handbook of Sensory Physiology, Vol. 1*, edited by W.R. Lowenstein (Springer-Verlag, Berlin), pp. 396-441.

Lewis, T.N. (**1994**). "Detection of scattered ambient noise by fish," Ph.D. Thesis, School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Lin, J., D.S. Faber, and M. Wood (**1983**). "Organized projection of the goldfish saccular nerve onto the Mauthner cell lateral dendrite," Brain Res. **274**, 319-324.

Lu, Z. and R.R. Fay (**1993**). "Acoustic response properties of single units in the torus semicircularis of the goldfish," J. Comp. Physiol. **173**, 33-48.

Moulton, J.M. and R.H. Dixon (**1967**). "Directional hearing in fishes," in *Marine Bio-Acoustics Vol. 2*, edited by W.N. Tavolga (Pergamon Press, New York), pp 187-232.

Pierce, A.D. (**1981**). *Acoustics* (McGraw-Hill, New York), pp. 44.

Platt, C. (**1977**). "Hair cell distribution and orientation in goldfish otolith organs," J. Comp. Neur. **172**, 283-298.

Popper, A.N. and P.L. Edds-Walton (**1995**). "Structural diversity in the inner ear of teleost fishes: Implications for connections to the Mauthner cell," Brain Behav. Evol. **46**, 131-140.

Rao, S.S. (**1990**). *Mechanical Vibrations* (Addison Wesley, Reading, Mass), pp. 146.

Rogers, P.H., A.N. Popper, M.C. Hastings, and W.M. Saidel (**1988**). "Processing of acoustic signals in the auditory system of bony fish," J. Acoust. Soc. Am. **83**, 338-349.

Rogers, P.H. and M. Cox (**1988**). "Underwater sound as a biological stimulus," in *Sensory Biology of Aquatic Animals*, edited by J. Atema, R.R. Fay, A,N. Popper, and W.N. Tavolga (Springer-Verlag, New York), pp 131-149.

Sand, O. and P.S. Enger (**1973**). "Evidence for an auditory function of the swimbladder in the cod," J. Exp. Biol. **59**, 405-414.

Sand, O., and A.D. Hawkins (**1973**). "Acoustic properties of the cod swimbladder," j. Exp. Biol. **58**, 797-820.

Schuijf, A. and R.J.A. Buwalda (**1975**). "On the mechanism of directional hearing in cod (*Gadus morhua* L.)," J. Comp. Phy

Sento, S. and T. Furukawa (**1987**). "Intra-axonal labeling of saccular afferents in the goldfish, *Carassuis auratus*: correlations between morphological and physiological characteristics," J. Comp. Neurol. **258**, 352-367.

von Frisch, K. (**1938**). "The sense of hearing in fish," Nature **141**, 8-11.

Webb, P.W. (**1986**). "Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (*Micropterus salmoides*)," Can. J. Fish. Aquat. Sci. **43**, 763-771.

Webb, P.W. and J.M. Skadsen (**1980**). "Strike tactics of *Esox*," Can. J. Zool. **58**, 1462-1469.

Zottoli, S.J. (**1997**). "Correlation of the startle reflex and Mauthner cell auditory responses in unrestrained goldfish," J. Exp. Biol. **66**, 243-254.